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Monokaryotic fruiting and its sexuality in self-fertile strains of *Filobasidiella neoformans* (*Cryptococcus neoformans*)

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Summary

Eight self-fertile strains (ATCC 28957, ATCC 34387, ATCC 34388, ATCC 34389, ATCC 34390, ATCC 34391, ATCC 34868, and ATCC 34876) of *Filobasidiella neoformans* produce monokaryotic hyphae, incomplete clamp connections, and basidia with basipetal chains of spores. ATCC 34868 produces heterothallic progeny, "α" and "a" self-sterile colonies, as well as self-fertile progeny. Four strains produce dikaryotic hyphae and complete clamp connections when paired with "a" mating-type strains. One produces dikaryotic hyphae and complete clamp connections when paired with "α" mating-type strains. Three do not produce dikaryotic hyphae and complete clamp connections with either "a" or "α" mating-type strains. The possible genetic controls of fruiting and sexual compatibility in the self-fertile strains are discussed.

The perfect state of *Cryptococcus neoformans* (Sanfelice) Vuillemin, a pathogenic yeast, is a Basidiomycete and is named *Filobasidiella neoformans* Kwon-Chung (1975). The fungus is described as a bipolar-heterothallic species, occurring in yeast cells in two mating types, "α" and "a". Alpha type appears to predominate in both clinical and natural isolates (Kwon-Chung and Bennett, 1978; Bowman and Ahearn, 1978).

Nonetheless, Shadomy and Utz (1966), Shadomy (1970), and Lurie and Shadomy (1971) observed clamped hyphae in 11 clinical isolates. The Coward and Stanford strains produced submerged and aerial hyphae on modified Sabouraud agar and the sporulation agar of Mrak et al. (1942). Cutler and Erke (1971) examined the clamps and septa of the Stanford strain by electron microscopy and found that septa were simple and without pores and that the clamplike structures were not fused with adjacent cells. Erke (1976) described the basidia and basidiospores produced by the Coward, Stanford, and C-145 strains and noted that a high percent of the clamps contained nuclei and that a majority of the hyphal cells were monokaryotic. Kwon-Chung (1978) noted that many hyphae of the Coward strain showed breakage at the septa with unfused clamps on one side. When she isolated single yeast cells from the Coward strain, Kwon-Chung found that two types of colonies developed: one type of colony was self-fertile and the other was self-sterile and of "α" mating-type. She concluded that although the self-

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fertile Coward isolate originally contained both "α" and "a" incompatibility factors, the "a" factor was lost during asexual multiplication.

This paper describes eight self-fertile strains that require independent genetic systems for fruiting and sexual compatibility respectively and a self-fertile strain that produces both "α" and "a" self-sterile colonies as well as self-fertile colonies.

Materials and Methods

Ninety-seven strains of *Cryptococcus neoformans* maintained lyophilized in the American Type Culture Collection (ATCC) were screened for self-fertility. Eight strains were self-fertile: ATCC 28957, ATCC 34387, ATCC 34388, ATCC 34389, ATCC 34390, ATCC 34391, ATCC 34868, and ATCC 34876. The historical information of each strain is listed in the fungus section of the 14th edition of the ATCC Catalogue of Strains (Jong, 1980).

Forty colonies, each derived from a basidiospore, were isolated from ATCC 34868 and were assigned monosporous strain numbers from 68-1 to 68-40. Each monosporous strain was examined for self-fertility and also mated with other monosporous strains. Two "a" mating-type strains, ATCC 28958 and ATCC 32608, and two "α" mating-type strains, ATCC 7472 and ATCC 32609, were used to detect mating ability of the self-fertile strains for formation of dikaryotic hyphae and complete clamp connections.

A 48 hr, yeast-malt extract slant-culture growth of each strain to be mated was washed with distilled water and the suspension was adjusted to a 2+ Wickerham reading with distilled water. Equivalent amounts of the yeast cell suspension to be screened and a suspension of one of the strains used to detect mating ability were combined and mixed with a Vortex mixer. A drop of suspension mixture was spread on duplicate mating agar plates (ATCC medium 1091). Cells on the agar surface were monitored for the development of clamped hyphae and basidia for 4 weeks. A drop of just the yeast cell suspension being screened was also spread on duplicate mating agar plates and the cells on the agar surface were monitored for self-fertility. Hay infusion agar (ATCC medium 320) and V-8 juice agar (ATCC medium 343), both modified with 4% agar, were also used to detect mating ability.

Nuclei were stained with aceto-iron-haematoxylin as described by Wittman (1962) and modified by F. Uecker (Personal communication) of the U.S. Department of Agriculture, Beltsville, Maryland. Cubes of agar containing hyphae, basidia, basidiospores, and yeast cells were cut from an agar plate and submerged in 1 : 3 acetic acid-absolute alcohol fixative for two days to two weeks. Wet mounts were dried at room temperature and fixed overnight in the acid-alcohol fixative.

After fixation, agar cubes and slides were washed in 95% and 70% ethanol, 15 min each to remove soluble materials. Specimens were treated in a mordant containing chrome alum, aluminum alum, and iodic acid. After mordanting, specimens were

hydrolyzed with a concentrated hydrochloric acid-absolute ethanol mixture for ten minutes. Specimens were hardened in fixative containing six parts absolute ethanol, three parts chloroform, and one part glacial acetic acid. Since the fixative also removed excess mordant and acid, it was necessary to replace the fixative after five, 15, and 60 min. After five minutes of hardening, the specimens could be stained.

Hyphae and basidia were teased from the agar cube in a concave slide containing McIlvaine (pH 4.4, 0.5 M phosphate) buffer. The teasing was best accomplished with insect pins under a dissecting microscope. The specimen was transferred to a slide and spread under a cover slip. Iron-haematoxylin solution was added at the edge of the cover slip and pulled through the specimen with a strip of filter paper placed at the opposite side. The buffer must be completely removed for good staining. Excess stain was removed by pressing the cover slip against the slide and the slide was heated gently over an alcohol flame.

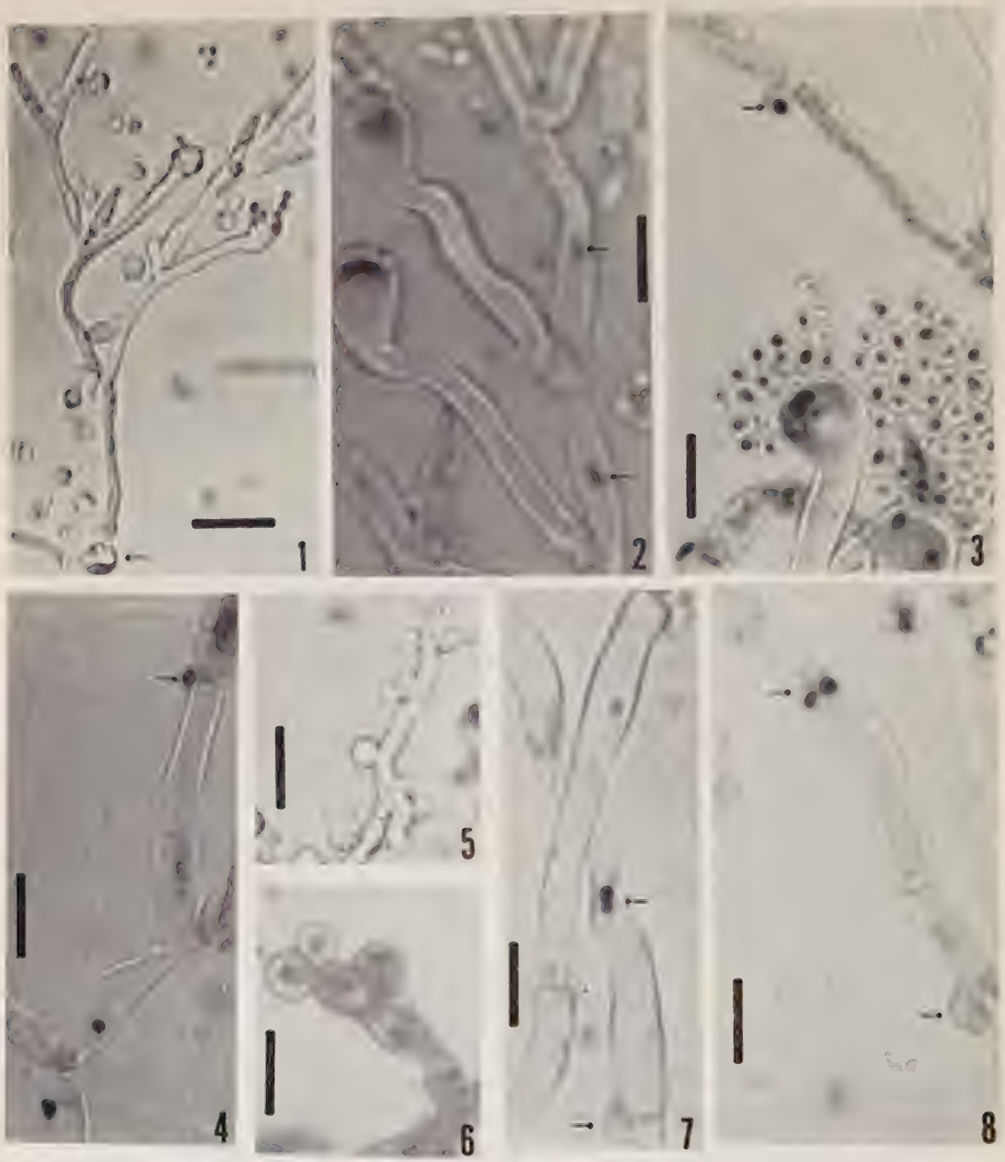
Results

The eight self-fertile strains produced clamped hyphae and basidia with spores on mating agar (MA), V-8 juice agar modified with 4% agar (V-8-M) and hay infusion agar modified with 4% agar (HI-M). Clamps produced by the self-fertile strains were incomplete. The clamps produced by mated strains were fused and complete. Nuclear staining detected dikaryotic hyphae throughout the mycelium of mated strains; nuclear staining of self-fertile strains detected monokaryotic hyphae throughout the mycelium, although basidia often contained two or more nuclei.

The ATCC 34868 is the most consistently productive self-fertile strain. It was received from Kwon-Chung in 1979 as NIH 371, an example of "α" mating-type and A serotype. The original isolate did not appear self-fertile; self fertility was noted after lyophilization. This strain produces large numbers of basidia with long chains of spores, usually within 48 hr, and on one occasion, fruiting occurred in yeast extract-malt extract broth (Fig. 1). The monokaryotic hyphae and uninucleate spores seen in Figures 2 and 3 are typical of this strain. Clamps do not fuse with adjacent cells and nuclei in the incomplete clamps are seen in Figures 3 and 4.

Of the 40 single basidiospore isolates from a self-fertile fruiting of ATCC 34868, 16 produce self-fertile colonies and incompatibility factors are detected in the other 24 (Tables 1 and 2). The data on mating competence of these 24 self-sterile strains are in agreement with the description of *Filobasidiella neoformans* as a biallelic bipolar species. Seven strains are "*a*" mating-type and 17 are "α" mating-type.

ATCC 28957, ATCC 34387, ATCC 34388, ATCC 34389, ATCC 34390, ATCC 34391, and ATCC 34876 produced true hyphae with clusters of blastospores (Figs. 5 and 6). Each of these self-fertile strains produced basidia with basidiospores although the number of basidia varied greatly with the strain, the type of medium, and the age of the



Figs. 1–4. Self-fertile strain ATCC 34868 of *Filobasidiella neoformans*. 1. Hyphae and basidia from a single cell (Scale: 20 μm). Note the single cell. 2. Monokaryotic hyphae (Scale: 10 μm). Note uninucleate cells. 3–4. Uninucleate basidiospores, monokaryotic hyphae, and incomplete clamps (Scale: 10 μm). Note nuclei in incomplete clamps.

Figs. 5–6. Blastospores of self-fertile *Filobasidiella neoformans*. 5. ATCC 28957 (Scale: 20 μm). 6. ATCC 34387 (Scale: 10 μm).

Figs. 7–8. Dikaryotic hyphae and complete clamps of mated strains of *Filobasidiella neoformans* (Scale: 10 μm). Note number of nuclei and complete clamps. 7. Cross between ATCC 34868 and ATCC 28958. 8. Cross between ATCC 34868 and ATCC 32608.

Table 1. Mating pattern produced by 24 monosporous strains of *Filobasidiella neoformans* isolated from self-fertile ATCC 34868

Monosporous Strain Number	68–1	68–3	68–5	68–7	68–8	68–12	68–15	68–16	68–22	68–24	68–27	68–28	68–29	68–30	68–36	68–37	68–38	68–6	68–9	68–10	68–25	68–26	68–35	68–39
68–1	–[a]																							
68–3	–	–																						
68–5	–	–	–																					
68–7	–	–	–	–																				
68–8	–	–	–	–	–																			
68–12	–	–	–	–	–	–																		
68–15	–	–	–	–	–	–	–																	
68–16	–	–	–	–	–	–	–	–																
68–22	–	–	–	–	–	–	–	–	–															
68–24	–	–	–	–	–	–	–	–	–	–														
68–27	–	–	–	–	–	–	–	–	–	–	–													
68–28	–	–	–	–	–	–	–	–	–	–	–	–												
68–29	–	–	–	–	–	–	–	–	–	–	–	–	–											
68–30	–	–	–	–	–	–	–	–	–	–	–	–	–	–										
68–36	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–									
68–37	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
68–38	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–							
68–6	+[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–						
68–9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–					
68–10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–				
68–25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–			
68–26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–		
68–35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	
68–39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–

[a] – indicates clamped hyphae and basidia were not produced.
[b] + indicates the production of clamped hyphae and basidia.

inoculum. Sometimes it was necessary to scrape the yeast from the agar medium to detect the hyphae and basidia obscured by the yeast colony. After this procedure, aerial hyphae bearing basidia with spores were rapidly produced. Usually dikaryotic fruiting is more vigorous than monokaryotic fruiting.

When ATCC 34868 was paired with "*a*" mating-type strains (e.g., ATCC 28958 and ATCC 32608), mating occurred; hyphae were dikaryotic and clamps were fused (Figs. 7 and 8). When ATCC 34868 was paired with "α" mating-type strains (e.g., ATCC 7472 and ATCC 32609), hyphae were monokaryotic and clamps did not fuse. ATCC 28957, ATCC 34387, and ATCC 34388 produced dikaryotic hyphae and fused clamps with "*a*" mating-type strains and monokaryotic hyphae and unfused clamps with "α" mating-type strains. ATCC 34391 (=Erke strain C-145) produced dikaryotic hyphae and fused clamps with "α" mating-type strains, but not with "*a*" mating-type strains. ATCC 34389, ATCC 34390, and ATCC 34876 did not produce dikaryotic hyphae and complete clamps with either "α" or "*a*" mating-type strains (Table 3).

Table 2. Mating types of 24 monosporous, self-sterile strains of *Filobasidiella neoformans* isolated from self-fertile ATCC 34868

Monosporous Strain Number	ATCC Accession Number																							
	α Mating-type 2505	2526	4189	4413	6352	7472	9506	24065	24066	32609	32735	32736	32738	34631	34878	34879	34880	34881	36480	36555	*a* Mating-type 28958	32608	34874	34875
α Mating-type																								
68–1	—[a]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+[b]	+	+	+
68–3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
68–38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
a Mating-type																								
68–6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
68–9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
68–10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
68–25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
68–26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
66–35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
68–39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—

[a] — indicates clamped hyphae and basidia were not produced.
[b] + indicates the production of clamped hyphae and basidia.

Table 3. Interactions of self-fertile strains and "*a*" and "α" mating-type strains of *Filobasidiella neoformans*

Self-fertile Strain	*a* Mating Type ATCC 28958 or ATCC 32608	α Mating Type ATCC 7472 or ATCC 32609
ATCC 28957	Dikaryotic, clamps completed	Monokaryotic, clamps incompleted
ATCC 34387	〃 〃 〃	〃 〃 〃
ATCC 34388	〃 〃 〃	〃 〃 〃
ATCC 34868	〃 〃 〃	〃 〃 〃
ATCC 34391	Monokaryotic, clamps incompleted	Dikaryotic, clamps completed
ATCC 34389	Monokaryotic, clamps incompleted	Monokaryotic, clamps incompleted
ATCC 34390	〃 〃 〃	〃 〃 〃
ATCC 34876	〃 〃 〃	〃 〃 〃

Discussion

Erke (1976) examined the basidia and the uninucleate basidiospores of several hyphae-forming strains. Single basidiospores, isolated by Erke, germinated to produce yeast cells, hyphae, basidia, and basidiospores. According to Erke (1976), this complete life cycle indicated primary homothallism, although the possibility of diploid basidiospores was not ruled out. The absence of fused clamps was noted by Cutler and Erke (1971) and Erke (1976). Although no explanation was given for this lack of fusion, Erke mentioned the similarity between the unfused clamps seen in self-fertile strains of *Filobasidiella neoformans* and the pseudoclamps described by Raper (1966) in other Basidiomycetes. These pseudoclamps occur when tetrapolar mating strains have a common *B* factor and different *A* factors or when there is an *A* factor mutation.

A factor mutants of *Coprinus lagopus* (Fries) Fries become morphological mimics of common-*B* heterokaryons and produce hyphae with pseudoclamps at most septa. These *A* factor mutants fruit regularly until the culture ages (Raper, 1966). Raper and Flexer (1971) described the result of mutations at the incompatibility loci of the tetrapolar species, *Schizophyllum commune* Fries. Mutation of the *B* factor renders the mutant strain compatible with all *B* factors and appears to convert tetrapolar incompatibility to bipolar incompatibility. Further mutation of the *A* factor of a *B* mutant causes the strain to appear homothallic since all incompatibility barriers are removed.

It is unclear whether pseudoclamps of *Coprinus lagopus* and the false clamps seen in self-fertile strains of *Filobasidiella neoformans* are similar. Mating factors in bipolar species appear to be a single genetic unit which controls the entire fruiting cycle (Raper, 1978). In tetrapolar species, two unlinked mating factors are responsible for the various functions such as nuclear pairing, clamp cell formation, conjugate nuclear division, clamp cell septation, and clamp cell fusion. Mutation at the single mating locus of a bipolar heterothallic strain could cause the strain to appear homothallic. The hyphae would be monokaryotic and fused clamps would not be necessary to separate dissimilar nuclei.

Raper (1978) defined primary homothallism differently than Erke (1976). The first requisite of a primary homothallic strain is that the fertile mycelium is homokaryotic. In a mycelium in which all nuclei are genetically similar, segregation and recominbation do not occur and the ability to bring together favorable properties of wild type strains is lost. Incompatibility factors cannot be detected in a primary homothallic strain. Secondary homothallism (Raper, 1978) involves incompatibility factors and self-fertility is the result of packaging opposite numbers of these factors in one spore (Dodge, 1927; Whitehouse, 1949). The fertile mycelium is heterokaryotic and recombination can occur. However, the benefits of outbreeding are only evidenced in true heterothallic strains.

A primary homothallic strain should not separate out incompatibility factors; since

the mycelium is homokaryotic. In a secondary homothallic strain, the yeast cells and basidiospores could appear uninucleate if they were diploid, but would the hyphae also appear to be uninucleate and diploid? The ATCC 34868 has monokaryotic hyphae (Figs. 2 and 3) and does separate out incompatibility factors (Tables 1 and 2). Instances of homothallic strains containing incompatibility factors are also found in *Rhodosporidium sphaerocarpum* Newell et Fell (1970) and *Sistotrema brinkmannii* (Bress.) J. Erikss. (Lemke, 1969).

The homothallic strain ATCC 22045 of *R. sphaerocarpum* produces heterothallic progeny. Its mycelia are uninucleate and the hyphae lack clamp connections. Teliospores, basidia, and sporidia are formed and sporidia mate with compatible sporidia produced by heterothallic strains. As in *F. neoformans*, one mating-type, survives better than the other. Newell and Fell (1970) claimed that the yeast cells of this homothallic strain were diploid, yielding diploid mycelia.

Erke (1976) suggested that the homothallic behavior of *F. neoformans* might be due to mutations at the mating loci. Kwon-Chung (1978) ruled out the possibility; except for the change from self-fertility to "α" mating-type. She also considered that the self-fertile strains of *F. neoformans* were possibly diploid. Instances of stable diploid strains have also been isolated in the laboratory in *Coprinus lagopus* and *Schizophyllum commune* (Casselton, 1965; Koltin and Raper, 1968; Mills and Ellingboe, 1969).

The existence of primary homothallic, bipolar heterothallic, and tetrapolar heterothallic patterns of sexuality in *Sistotrema brinkmannii* is well recognized (Biggs, 1937; Lemke, 1969; Ullrich, 1973; Ullrich and Raper, 1975). Lemke (1969) provided considerable evidence suggesting that spores from homothallic strains of *S. brinkmannii* were uninucleate and haploid. However, Ullrich (1973) indicated that the uninucleate spores were not necessarily haploid and that the possibility of aneuploidy or duplication of a chromosomal segment containing the incompatibility factor of heterothallic forms should not be eliminated. Lemke (1969) also reported that some homothallic strains might possess at least some part of an incompatibility factor; thus homothallic and bipolar strains might occassionally be hybridized through nutritional forcing. As in *S. brinkmannii*, the existence of incompatibility in the self-fertile strains of *Filobasidiella neoformans* suggests that the extant homothallic strains may have been derived from the bipolar heterothallic strains through mutations (including loss) in the incompatibility factor.

Although incompatibility controls various morphogenetic steps from fusion of monokaryons to establishment of the dikaryon, another gene is responsible for the initiation of fruiting in several species of Basidiomycetes in both sexual and asexual cycles (Stahl and Esser, 1976; Esser, 1977; Esser and Meinhardt, 1977). In dikaryotic fruiting complete clamp connections guarantee the equal distribution of parental nuclei during mycelial growth until karyogamy and meiosis occur. In monokaryotic fruiting karyogamy and meiosis do not occur, only mitosis. Compatible mating types are necessary for a sexual

cycle but monokaryotic fruiting can be initiated by a threshold gene. In the dikaryon at least one of the nuclei must carry this gene for fruiting to occur even under the most ideal environmental conditions. Recently monokaryotic fruiting has also been reported in the bipolar incompatible Ascomycete *Podospora anserina* (Ces.) Rehm (Esser and Graw, 1980). This hypothesis is also applicable to explain the phenomenon of monokaryotic fruiting seen in *Filobasidiella neoformans* because the basidiospores are formed on monokaryotic hyphae with incomplete clamp connections, in which nuclear exchange, karyogamy and meiosis are blocked by an incompatibility gene. The significance of this threshold gene to dikaryotic fruiting in *F. neoformans* is unknown.

In addition, the inability of 3 of the 8 self-fertile strains to produce the complete clamp connections with any mating-type strain suggests the possibility of additional mating-types within the complex.

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Notes on some Japanese Ascomycetes XVI*

Shun-ichi UDAGAWA** and Tetsuo MUROI***

宇田川俊一**・室井哲夫***：日本産子のう菌類知見（XVI）*

Summary

This paper presents descriptions of the following seven ascomycetous fungi isolated from soil and cow dung: *Apiosordaria microcarpa* Udagawa & Muroi sp. nov. (Sordariaceae) from paddy soil, Toyama; *Chaetomium amygdalisporum* Udagawa & Muroi sp. nov. (Chaetomiaceae) from soil, Gunma; *Chaetomium citrinum* Udagawa & Muroi sp. nov. from paddy soil, Tochigi; *Kernia retardata* Udagawa & Muroi sp. nov. (Microascaceae) from paddy soil, Tochigi; *Pteridiosperma lenticularis* Udagawa & Muroi sp. nov. (Melanosporaceae) from cow dung, Tochigi; *Farrowia malaysiensis* D. Hawksw. (Chaetomiaceae) from soil, Kagoshima; and *Preussia fleischhakii* (Auersw.) Cain (Sporormiaceae) from river sludge, Wakayama. As an additional note, the correct taxonomic placement of *Chaetomium piluliferoides* Udagawa & Horie is discussed.

In this, the sixteenth paper of the series, seven fungal species isolated from soil and cow dung are described and illustrated below. Among them, five are proposed as new species, while *Farrowia malaysiensis* D. Hawksw. and *Preussia fleischhakii* (Auersw.) Cain are reported from Japan for the first time. Color terms used in the descriptions are taken from two sources, namely "Guide to Colour Standard (6th ed.)" by Nippon Shikisai Kenkyujo (1964) and "A Mycological Colour Chart" by R. W. Rayner (1970). Type specimens and living cultures of the species have been maintained in the Mycological Herbarium and Culture Collection, National Institute of Hygienic Sciences, Tokyo, Japan (NHL).

Apiosordaria microcarpa Udagawa & Muroi, sp. nov. (Fig. 1)

Stat. anam. *Chrysosporium.*

Peritheciis sparsis vel plerumque in catervis parvis aggregatis, superficialibus, atribrunneis vel fere nigris, pyriformibus vel elongatis, 180–250×115–160 μm, in parte inferiore pilosis; pilis longis, flexuosis, brunneis, simplicibus, levibus, septatis, 2.0 2.5(–3.5) μm diam. Collo perithecii atribrunneo vel nigro, conico vel elongato, 40–65×70 -95

* XV: this transaction, **18**: 302-311, 1977.

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μm, superne amplificato, late ostiolato, cum tuberculis dense aggregatis, dilute flavo-brunneis praedito. Peridio aliquantum crasso, membranaceo, multistrato; cellulis externis complanatis, brunneis, incrassatis, angularibus, 4.0–10.0 μm diam et cellulis interioribus hyalinis, angularibus. Ascis octosporis, late clavatis, 65–90 × 18–22 μm, superne plus minusve truncatis, ad apicem cum annulo simplici praeditis, 2.0–2.5 μm diam, brevi-stipitatis, postremo evanescentibus. Paraphysibus hyalinis, filiformibus vel ventricosis, septatis, 4.0–5.0 μm diam. Ascosporis biseriatis, primum hyalinis, guttulatis, unicellularibus, ellipsoideis, deinde transverse uniseptatis, ad septum parum constrictis, totaliter (18–)20–22.5(–24) μm longis; cellula superiore profunde olivaceo-brunnea vel atribrunnea, ellipsoidea, basi truncata, 15–19 × 10–12.5 μm, ad apicem aliquantum

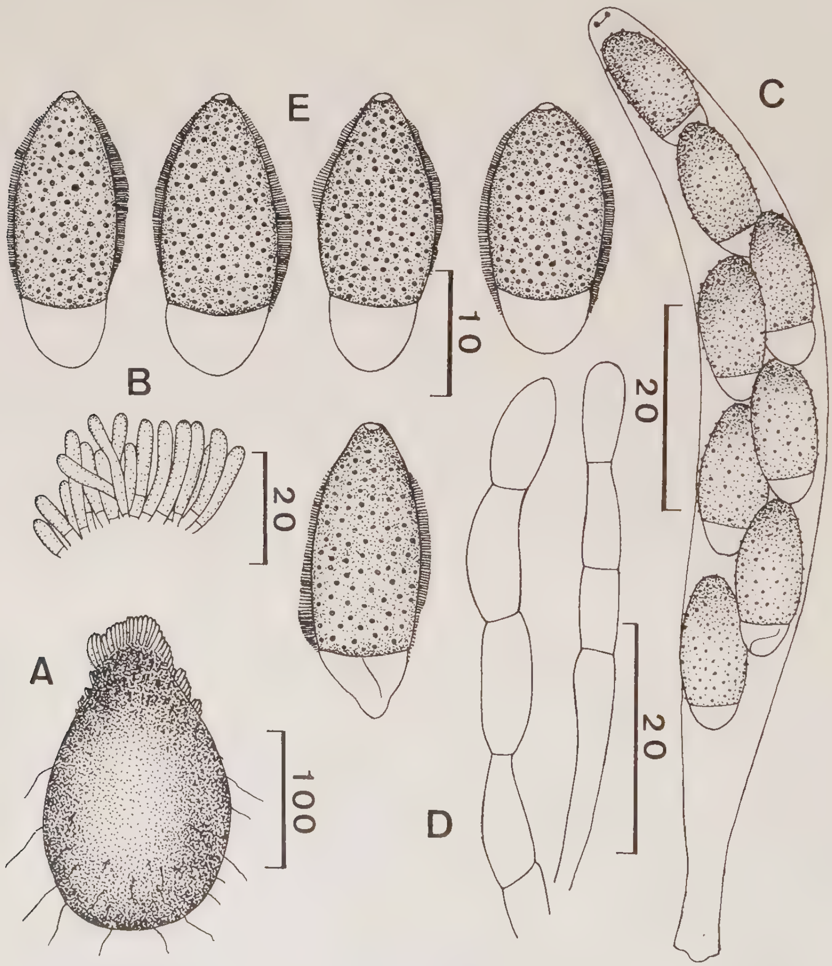


Fig. 1. *Apiosordaria microcarpa*, NHL 2872. A. Perithecium. B. Tubercules in upper part of the perithecium. C. Ascus. D. Paraphyses. E. Ascospores. (All arabic numerals in scales denote μm.)

anguste acuta, cum parie spinosis, circa 1.0 μm crassis vestita, cum poro germinali hyalino, apicali, circa 1.0 μm diam praedita; cellula inferiore hyalina vel dilute brunnea, cylindracea, ad apicem late rotundata, 5–6.5 × 7.5–9 μm, plerumque collapsa. Appendice gelatinosa nulla.

Mycelio ex hyphis profunde brunneis, ramosis, septatis, levibus, 1.0–3.0 μm diam composito, saepe fasciculato. Conidiis holoblasticis, simplicibus, singularibus, dispersis in hypha, fere sessilibus, ovatis vel clavatis, circa 2.5–6.0 μm longis et 1.5–2.5 μm diam, basi truncatis, superne rotundatis, dilute flavo-brunneis, levibus.

Coloniis in agaro 'oatmeal' ad 23 C effusioribus, velutinis vel floccosis, olivaceo-griseis vel profunde olivaceo-griseis; peritheciis abundantibus; reverso olivaceo-nigro vel nigro.

Holotypus—isolatus e solo sativi (*Oryza sativa*), Toga-mura, Higashi-tochinami-gun, Toyama, in Japonia, 30.ix.1978, legit. S. Udagawa, No. 2872. In Herbario NHL.

Etymology: *microcarpa* = small fruited, referring to the small size of the perithecia.

At 37 C, growth is very slow, and perithecia usually are not formed.

The distinctive features of this *Apiosordaria* are its smaller perithecia, aggultinated tubercles of perithecial neck, 8-spored asci and smaller ascospores. It resembles *A. yaeyamensis* Morinaga et al. (1979), especially in having 8-spored asci, but differs obviously in the small size of perithecia whose upper portions are covered with swollen tubercles, in the short length of its asci, in its larger ascospores and in the presence of an anamorphic state.

Chaetomium amygdalisporum Udagawa & Muroi, sp. nov. (Figs. 2, 7, 8)

Stat. anam. *Phialophora*.

Peritheciis superficialibus, sparsis vel laxe aggregatis, atribrunneis vel nigris, obovatis, (160–)180–240 × (120–)140–180 μm, late ostiolatis, ad substratum cum rhizoideis sparsis affixis. Peridio perithecii tenui, membranaceo. Pilis terminalibus abunde effectis, densis, profunde olivaceo-brunneis vel atribrunneis, sinuatis vel laxe spiralibus, cum circa 20 convolutis instructis, non ramosis, septatis, 400–650 × 2.5–3.0 μm, basi usque 4.0–4.5 μm diam, subtiliter asperis, ad apicem leviter acutis gradatim attenuatis. Pilis lateralibus cum iis terminalibus similaribus sed brevioribus et leniter sinuatis. Ascis octosporis, cylindraceis, 85–100 × 11–14 μm (pars sporif. 72–86 μm longis), superne rotundatis, brevi-stipitatis, evanescentibus. Ascosporis uniseriatis, olivaceo-brunneis vel profunde olivaceo-brunneis, amygdaliformibus, interdum cuneatis vel pyriformibus, 13–16(–17.5) × 9–11.5 μm, uniporosis; poro germinali apicali, circa 1.0 μm diam.

Mycelio e hyphis hyalinis, ramosis, levibus, septatis, 1.0–5.0 μm diam composito. Conidiophoris simplicibus, brevibus, hyalinis, levibus, septatis. Cellulis conidiogenis (monophialidibus) integris, terminalibus, determinatis, cylindraceis, vulgo 5–20 × 1.5–4.0 μm, levibus. Conidiis hyalinis, parvis, bacilliformibus, 2.5–3.5 × 1.5–1.8 μm, levibus, in globis viscosis 6.0–10.0 μm diam aggregatis.

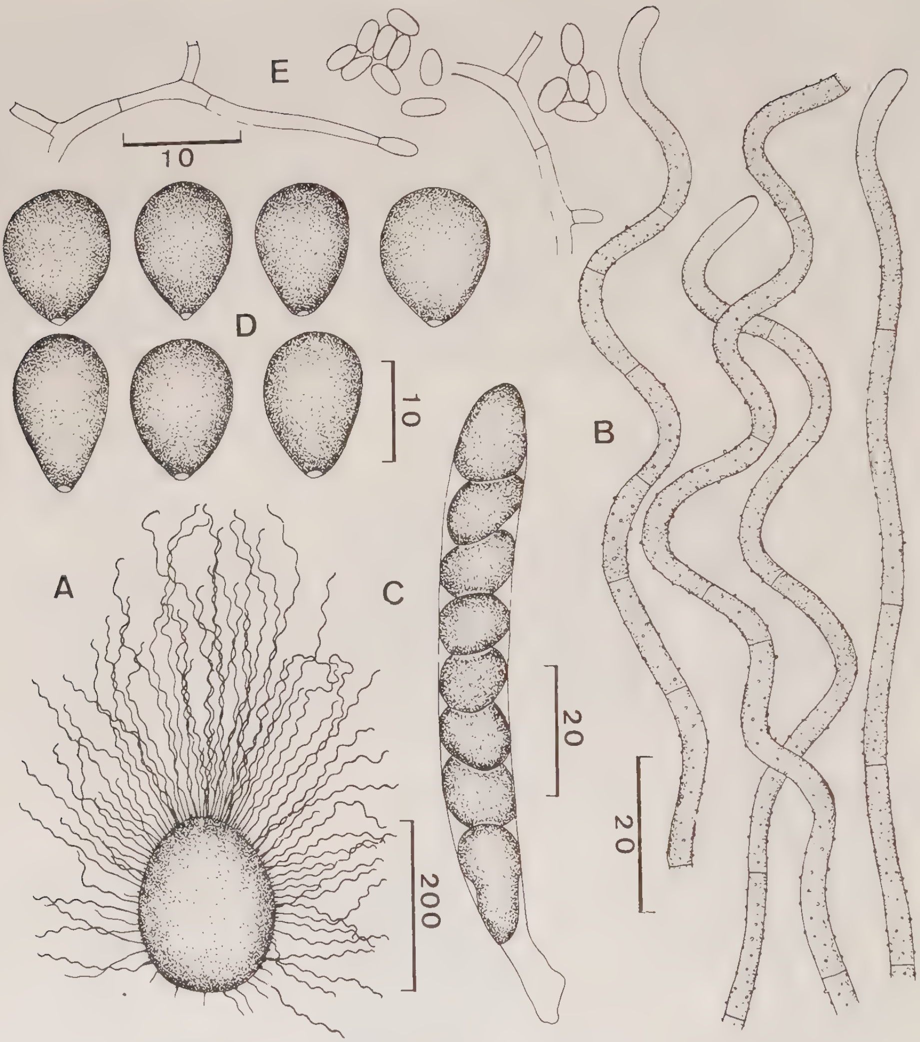


Fig. 2. *Chaetomium amygdalisporum*, NHL 2874. A. Perithecium. B. Terminal hairs. C. Ascus. D. Ascospores. E. Conidia. (All arabic numerals in scales denote μm.)

Coloniis in agaro 'cellulose' ad 37 C effusis, plus minusve floccosis, cum mycelio aerio laxo, griseis, 'Greenish grey–Olivaceous grey–Dull green (Rayner, 1970)'; peritheciis et conidiis abundantibus; reverso incolorato.

Holotypus—isolatus e solo, Kiryû-shi, Gunma, in Japonia, 3.v.1978, legit. S. Udagawa, No. 2874. In Herbario NHL.

Etymology: *amygdalisporum*=almond-shaped spores, referring to the shape of ascospores.

At 23 C, growth-rate is 1/2 to 1/3 of that at 37 C, with perithecial production very reduced, and almost conidial.

Table 1. Temperature and media effects on the growth rates of *C. amygdalisporum*

Medium	Growth rate at 10 days*					
	15 C	23 C	37 C	45 C	50 C	55 C
PCA	0	28	>85	73	37	0
Oatmeal agar	0	–	>85	>85	45	0
Cellulose agar	0	54	>85	–	–	–

* Average diam of three colonies (mm).

Chaetomium mollicellum Ames (1961 [1963]), the only closely related species of the '*C. crispatum*-Artengruppe' of Dreyfuss (1975), differs in possessing long straight (or wavy) terminal hairs and smaller (8–10×5–7 μm) globose-ovate ascospores, and in the absence of an anamorphic state. During work on the screening of mycotoxin-producing Chaetomia, two secondary metabolites of *C. amygdalisporum*, neocochliodinol and mollicellin G were isolated from a rice culture (Sekita et al., 1981). Mollicellins A–H, a series of fungal depsidones, are the major products of *C. mollicellum* and their mutagenicity and antibacterial activity were reported in *Salmonella*/microsome tests by Stark et al. (1978). The production of mollicellin G by *C. amygdalisporum* suggests an even more intimate relationship between both fungi.

Chaetomium amygdalisporum is thermotolerant rather than thermophilic (Table 1) and its optimal growth was reached at 37 C.

Chaetomium citrinum Udagawa & Muroi, sp. nov. (Figs. 3, 9)

Peritheciis atribrunneis vel fere nigris, globosis vel subglobosis, 280–425 μm diam, ostiolatis, ad substratum cum rhizoideis dilute brunneis affixis. Peridio perithecii tenui, membranaceo, rubenti-brunneo. Pilis terminalibus flavo-brunneis vel brunneis, gracilibus, irregulariter undulatis, interdum geniculatis, usque 650 μm longis, basi 3.5–6.0 μm diam, ad medium 2.0–2.5(–3.0) μm diam, non ramosis vel interdum ramosis, septatis, leviter asperis, numerosis, supra dense implicatis, cum capitulo flavo-viridi et fruticoso compositis. Pilis lateralibus dilute flavo-brunneis vel dilute brunneis, rectis vel plus minusve flexuosis, usque 200–220 μm longis, basi 4.0–5.0 μm diam, subtiliter asperis, septatis, ad apicem rotundatis gradatim attenuatis. Ascis octosporis, clavatis, 52–70×10–12 μm (pars sporif. 25–32 μm), brevi-stipitatis, evanescentibus. Ascosporis biseriatis, profunde olivaceo-brunneis, formis variis ornatis, subglobosis, ovatis, ellipsoideis, reniformibus, cordatis, vel triangularibus, (6.0–)7.0–12.5(–16.0)×4.5–6.5(–7.0) μm, saepe inaequilateris, utrinque apiculatis vel uno apice rotundatis et alio apiculatis, uniporosis; poro germinali apicali, circa 1.0 μm diam. Conidiis incognitis.

Coloniis in agaro 'cellulose' ad 23 C effusis, tenuibus, planis, flavo-brunneis, 'Citrine (Rayner, 1970)', cum rubenti colore suffusis; peritheciis abundantibus; reverso incolorato vel dilute aurantiaco; in agaro 'oatmeal' ad 23 C effusis, aliquantum floccosis, dilute

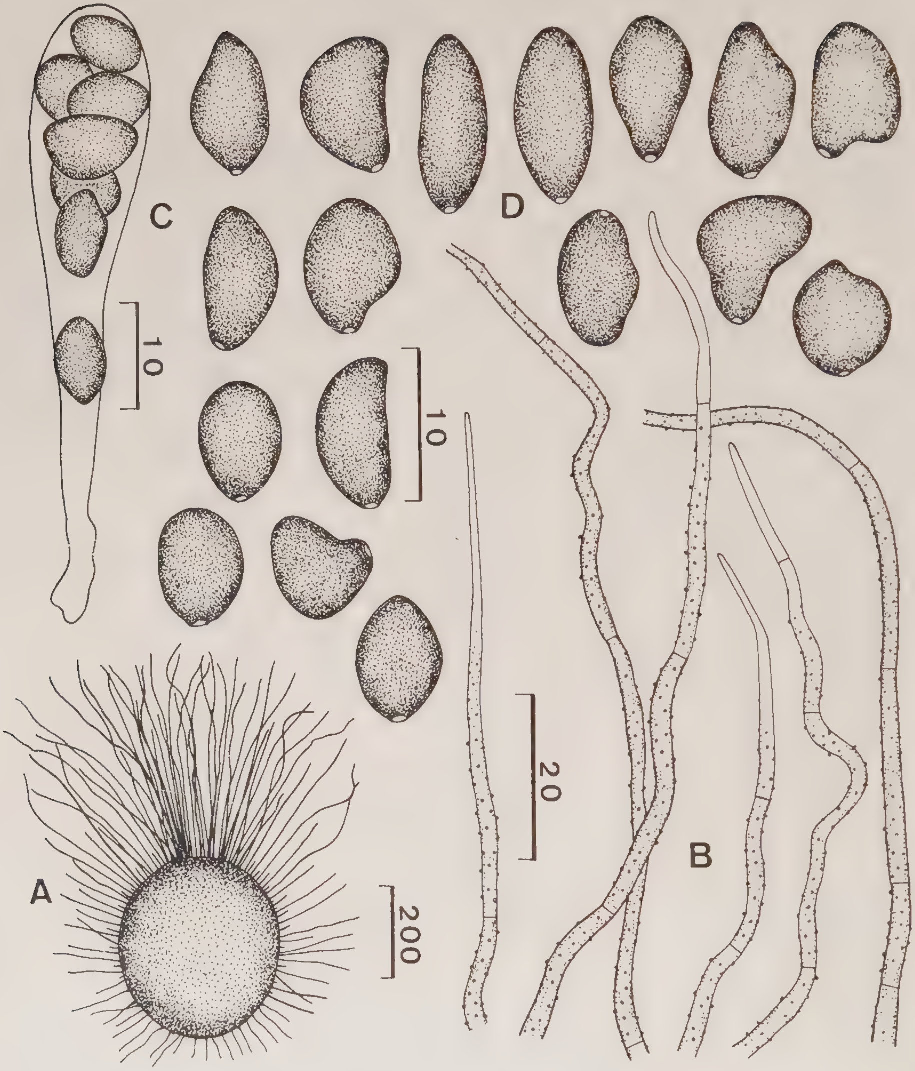


Fig. 3. *Chaetomium citrinum*, NHL 2873. A. Perithecium. B. Terminal hairs. C. Ascus. D. Ascospores. (All arabic numerals in scales denote μm.)

olivaceis, 'Olivaceous buff Greenish olivaceous (Rayner, 1970)'; peritheciis abundantibus; reverso brunneo-griseo.

Holotypus—isolatus e solo sativi (*Oryza sativa*), Nishinasuno-machi, Nasu-gun, Tochigi, in Japonia, 23.iv.1978, legit. T. Muroi, No. 2873. In Herbario NHL.

Etymology: *citrinum*=citrine color of colony surface and perithecial heads.

At 37 C, growth is nil.

Chaetomium citrinum is characterized by the yellowish color of its massed perithecia

which are influenced by the formation of bushy heads, long undulate and sometimes geniculate terminal hairs, and particularly by its irregular-shaped ascospores. The species is believed to be more closely related to *C. nozdrenkoae* Sergejeva (1961) and *C. irregulare* Sörgel ex Gams (1966) than to other members which produce irregular-shaped ascospores in the genus. It differs from both of the latter species principally in the nature of its terminal hairs or its smaller asci and ascospores.

Farrowia malaysiensis D. Hawksworth, Persoonia **8**: 178. 1975. (Figs. 10–12)

Perithecia superficial, scattered, dark olive brown to nearly black, vase-shaped, (145–)160–220 × (55–)60–85 μm, hairy, attached firmly to substrate with well-developed rhizoids; neck cylindrical, 55–120 × 25–40 μm, ostiolate, composed of a beak of fused terminal hairs; peridium thin, membranaceous, brown to dark brown, outer layer composed of brownish, angular cells measuring 7.0–14.0 × 5.0–11.0 μm. Terminal hairs arising synchronously from adjacent elongate cells at the neck of perithecium, brown to dark brown, straight, 95–200(–280) × 4.0–6.0 μm, 3.0–3.5 μm wide at the middle, unbranched or sometimes branched at right angles, tapering and fading above toward the rounded tip, smooth-walled. Lateral hairs similar to the terminal hairs but fewer in number, straight, 45–90 × 2.0 3.0 μm near the base, unbranched, septate, arising singly from the peridium. Asci borne in a basal fascicle, 8-spored, clavate, 30–40 × 11–12 μm (pars sporif. 24–28 μm), short-stipitate, evanescent. Ascospores biseriate, olive brown to dark brown, lemon-shaped, 7.0–9.0 × 7.0–7.5 × 5.0–5.5 μm, smooth-walled, apiculate at both ends, with a single apical germ pore measuring about 1.0 μm in diam. Conidiophores micronematous or semimacronematous, mononematous. Conidiogenous cells monoblastic, integrated, terminal, determinate, cylindrical. Conidia singly borne on the conidiogenous cells, hyaline, globose, clavate, or pyriform, 9.0–12.0(–14.0) × 7.5–9.0 μm, slightly truncate at the base, smooth-walled.

Colonies on oatmeal agar spreading broadly, floccose, somewhat zonate by abundant formation of perithecia, dark olive gray to nearly black with a reddish brown tint; reverse gray to dark gray with reddish brown color.

At 37 C, growth is better than that at 23 C and there is abundant perithecial production.

Isolation: in cultures from soil, Nakatane-machi, Kumage-gun, Kagoshima, Japan, Dec. 1958, col. S. Udagawa, No. 2260, NHL.

Specimens examined: culture received from Commonwealth Mycological Institute, IMI 183184.

Re-examination of the Japanese strain (NHL 2260), which at one time was regarded as representing *Chaetomium torulosum* Bain. (Udagawa, 1960), and detailed comparisons with the type strain indicate the need to correct the previous report. The terminal hairs and ascospores of *F. malaysiensis* are very similar to those of *Chaetomium udagawae* Serg.

ex Udagawa but the latter species differs in having coarsely roughened terminal hairs which do not arise synchronously from the adjacent elongated cells at the apex of the perithecia and are not fused to form a neck, and in producing sterigmatocystin as the toxic secondary metabolite (Udagawa et al., 1979).

To our knowledge, this is the first report of *F. malaysiensis* outside of Malaysia.

Kernia retardata Udagawa & Muroi, sp. nov. (Figs. 4, 13, 14)

Stat. anam. *Scopulariopsis.*

Cleistotheciis superficialibus, sparsis vel in catervis parvis aggregatis, nigris, nitidis, globosis vel subglobosis, 130–250 μm diam, non ostiolatis, glabris vel parum pilosis; pilis brevibus, hyalinis, septatis, levibus, usque 60 μm longis, basi 5.0–7.0 μm diam. Peridio perithecii plerumque 5–32 μm crasso, profunde olivaceo-brunneo, opaco, plus minusve coriaceo, pseudoparenchymato, 'textura globulosa' vel 'textura angularis', bistrato; strato externo ex cellulis olivaceo-brunneis, incrassatis, rotundatis a latere visis, 5–12 μm diam composito; strato interiore ex cellulis hyalinis, tenuibus, angularibus, complanatis a latere visis, 12–25 μm diam composito. Ascis octosporis, globosis vel subglobosis vel ovoideis, 11–15 μm diam, non stipitatis, evanescentibus, irregulariter dispositis. Ascosporis primum dextrinoideis, stramineis vel dilute flavo-brunneis, in massa flavo-aurantiacis vel rubro-brunneis, unicellularibus, ellipsoideis vel raro triangularibus, asymmetrice planis a latere visis, 4.5–7.0×3.5–5.0 μm, ad extremum aliquantum acutis, levibus, 'de Bary bubble' praeditis, uniporosis.

Mycelio e hyphis hyalinis, ramosis, septatis, levibus, 1.5–3.0 μm diam, saepe funiculosis composito. Conidiophoris simplicibus vel superne ramosis et penicillatis, erectis, brevibus, 5–12×3.0–4.5 μm, levibus. Cellulis conidiogenis (annellophoris) interdum singularibus et in conidiophoris incorporatis, sed saepe 4–6 verticillatis in conidiophoris brevibus, in penicillis compactis, percurrentibus, lageniformibus, 8–12×2.5–4.0 μm; parte annellata saepe elongata, usque 18 μm longa, 1.5–2.5 μm diam. Conidiis breviter catenatis, hyalinis, in massa albidis, ellipsoideis vel pyriformibus vel ovatis, 3.5–7.0(–10.0) ×2.0–4.0 μm, basi truncatis, plerumque levibus.

Coloniis in agaro 'cellulose' ad 23 C restrictis, tenuibus, modice sulcatis, plus minusve funiculosis, primum albis conidiis visis, deinde parum griseis et cleistotheciis nigris effectis; in agaro cum 'potato-carrot' ad 23 C conspicue restrictis, tenuibus, velutinis vel funiculosis, griseis, 'Pale mouse grey -Mouse grey (Rayner, 1970)'; cleistotheciis et conidiis abundantibus; reverso 'Pale luteous–Buff (Rayner, 1970)'.

Holotypus—isolatus e solo sativi (*Oryza sativa*), Nishinasuno-machi, Nasu-gun, Tochigi, in Japonia, 28.v.1978, legit. T. Muroi, No. 2879. In Herbario NHL.

Etymology: *retardata*=delayed, referring to its retarded growth on common media. At 37 C, there is only very slight growth.

The genus *Kernia* was proposed by Nieuwland and currently 9 species have been

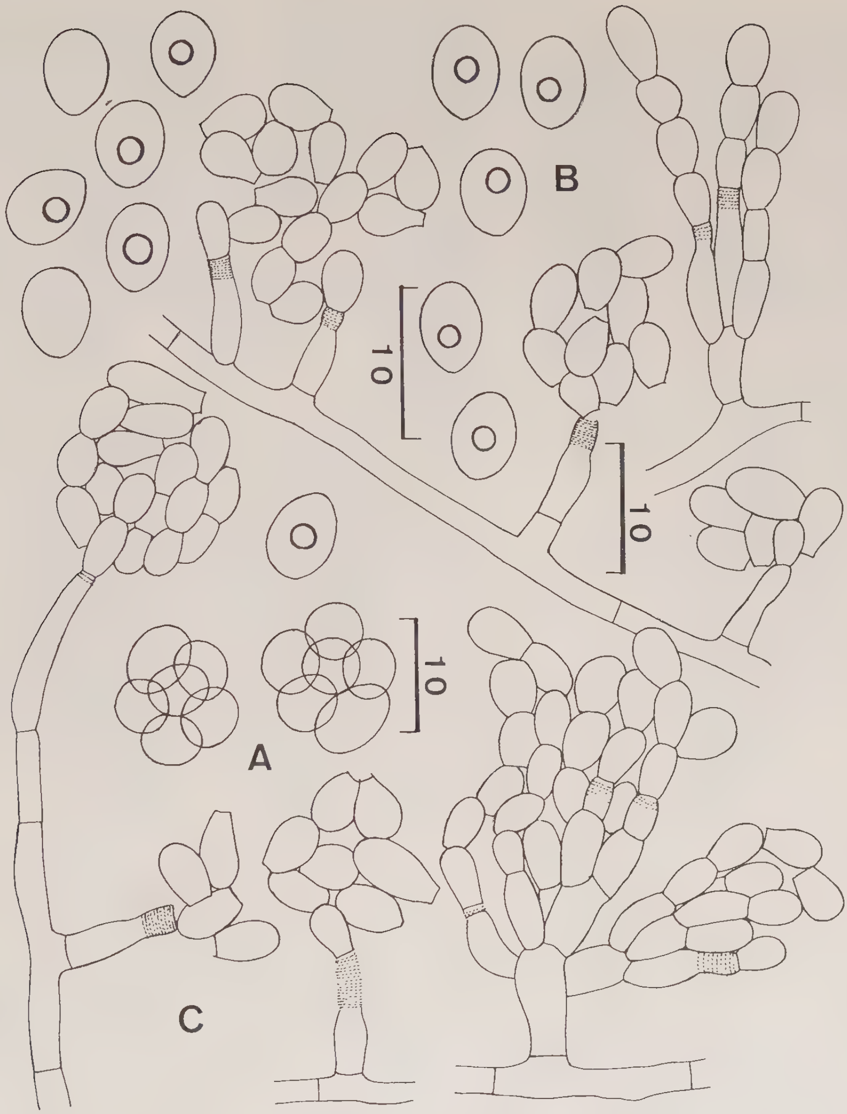


Fig. 4. *Kernia retardata,* NHL 2879. A. Asci. B. Ascospores. C. Conidia. (All arabic numerals in scales denote μm.)

recognized (Malloch and Cain, 1971, 1973; Lodha, 1971; Locquin-Linard, 1977, 1980). Among these, *K. retardata* is related to *K. pachypleura* Malloch & Cain and *K. hyalina* Malloch & Cain. The ascospores of *K. retardata* and *K. pachypleura* have a similar size range; the spores of *K. pachypleura* strains, IMI 151 091 (cf Malloch and Cain) and NHL 78-S-44 (cf Udagawa and Muroi) are (4.5–)5.5–7.0 × (3.5–)4.0–5.0 μm. *Kernia pachypleura* is readily separable from the present fungus on the basis of its ascospores which contain two germ pores and its markedly dark-colored conidia.

Kernia retardata seems to resemble *K. hyalina* more closely than *K. pachypleura* because *K. hyalina* possesses pale-colored ascospores with one germ pore. However, *K. retardata* is distinguished by its larger asci, ascospores and conidia, the yellowish brown to yellowish orange color of its massed ascospores, and its white massed conidia. From studies of the ATCC 22155 strain, it was found that *K. hyalina* grew more luxuriantly at 37 C than *K. retardata.*

Kernia ovata (Booth) Malloch & Cain is also close to *K. retardata* but differs in having slightly larger ascospores with two prominent germ pores and in lacking a *Scopulariopsis* anamorph.

Preussia fleischhakii (Auersw.) Cain, Can. J. Bot. **39**: 1640. 1961. (Figs. 5, 15)

≡*Sporormia fleischhakii* Auersw., in Rabenh. Fung. eur. 921. 1866 and Hedwigia **7**: 66. 1868.

=*Fleischhakia laevis* Auersw., Hedwigia **8**: 2. 1869.

Cleistothecia scattered or loosely aggregated, mostly superficial, black, subspherical, 385–700 μm in diam, glabrous or often covered with loose aerial hyphae, nonostiolate. Peridium dark brown, thin, membranaceous; outer layer 'textura intricata'; inner layer 'textura angularis', composed of hyaline, isodiametric, angular cells measuring 5.0–8.0×4.0–6.0 μm. Asci 8-spored, bitunicate, broadly clavate, (48–)60–70(–80)×22–28 μm, broadly rounded above, short-stipitate, with crozier at the base; pseudoparaphyses hyaline, filiform, septate, about 2.0 μm in diam. Ascospores multiseriate, olivaceous brown to dark brown, four-celled, nearly cylindrical, 30–37×6.0–7.5 μm, straight or slightly curved, transversely septate, deeply constricted at the septa, smooth-walled; segments easily separable; terminal cells elliptical to ovate, more or less narrowing toward the ends, 8.0–11.0×6.0–7.5 μm; mid-cells ovate to subglobose, 6.0–8.0(–8.5)×5.5–7.0 μm; germ slit parallel or slightly oblique; gelatinous sheath hyaline, thin.

Colonies on oatmeal agar growing fairly rapidly, floccose to somewhat funiculose, black-dotted with groups of cleistothecia throughout the surface, gray with a pale red purple tint; reverse grayish red purple.

At 37 C, growth is nil.

Isolation: in culture from river sludge, Wakagawa, Wakayama-shi, Wakayama, Japan, Feb. 1976, col. N. Toyazaki, No. 2802, NHL.

Preussia fleischhakii is most similar to *P. funiculata* (Preuss) Fuckel, but differs in having shorter asci and transversely septate ascospores.

Pteridiosperma lenticularis Udagawa & Muroi, sp. nov. (Figs. 6, 16–19)

Stat. anam. *Phialophora.*

Peritheciis superficialibus, sparsis vel in catervis parvis aggregatis, clare flavis vel aurantiaco-flavis, typice ampulliformibus, 300–400×270–335 μm, in parte inferiore

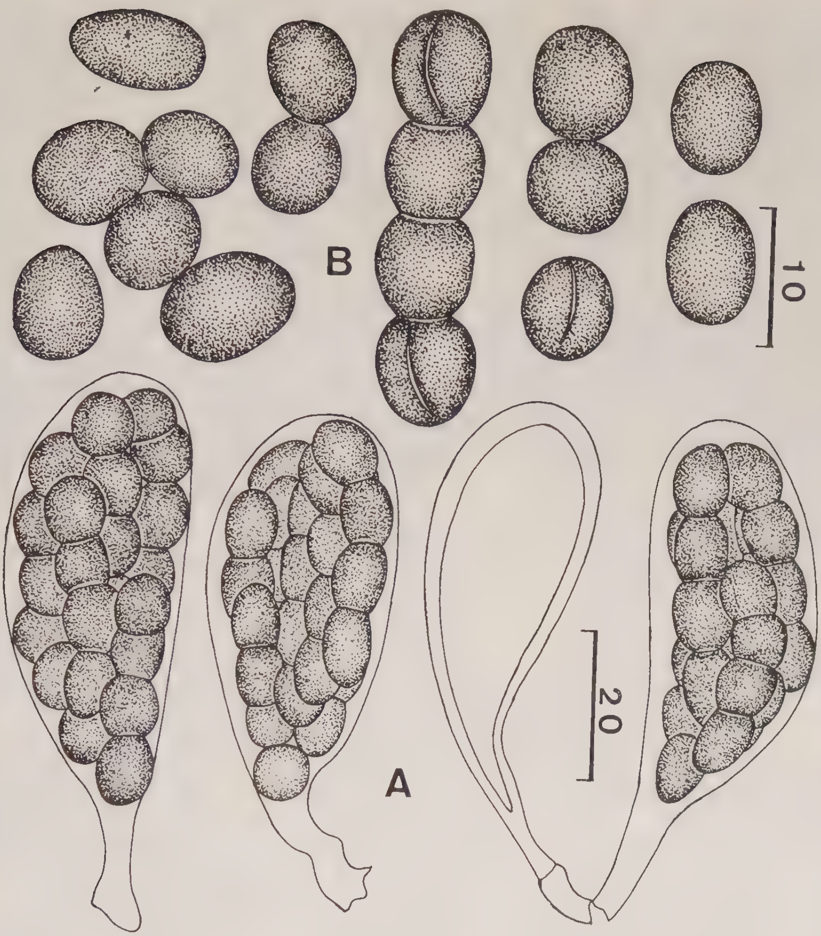


Fig. 5. *Preussia fleischhakii*, NHL 2802. A. Asci. B. Ascospores. (All arabic numerals in scales denote μm.)

pilosis; pilis hyalinis vel dilute flavis, flexuosis, simplicibus, septatis, levibus vel parum asperis, usque 160–220 μm longis et basi 5–9 μm diam. Collo perithecii conico vel brevicylindraceo, 55–120 × 80–105 μm, cum ostiolo rotundo et ciliato praedito; pilis numerosis, rectis, remote septatis, simplicibus, dilute flavis, usque 95–120 μm longis, 3.5–8.0 μm diam, levibus, incrassatis, ad apicem angustatis et rotundatis, interdum successive prolificantibus. Peridio perithecii dilute flavo, membranaceo, semitranslucenti; strato externo ex cellulis grandibus, dilute flavis, aliquantum incrassatis, angularibus, 10–32 × 6 26 μm composito; strato interiore ex cellulis hyalinis, grandibus, tenuibus, rotundatioribus composito. Ascis octosporis, late clavatis, (55–)80–100 × (22–)24–30 μm, superne late rotundatis, sine annulo incrassato, brevi-stipitatis, valde evanescentibus, in fasciculo basi dispositis. Paraphysibus indistinctis, hyalinis, moniliformibus, 8–14 μm diam. Ascosporis biseriatis vel irregulariter stipatis, unicellularibus, lenticularibus, late ellipsoideis

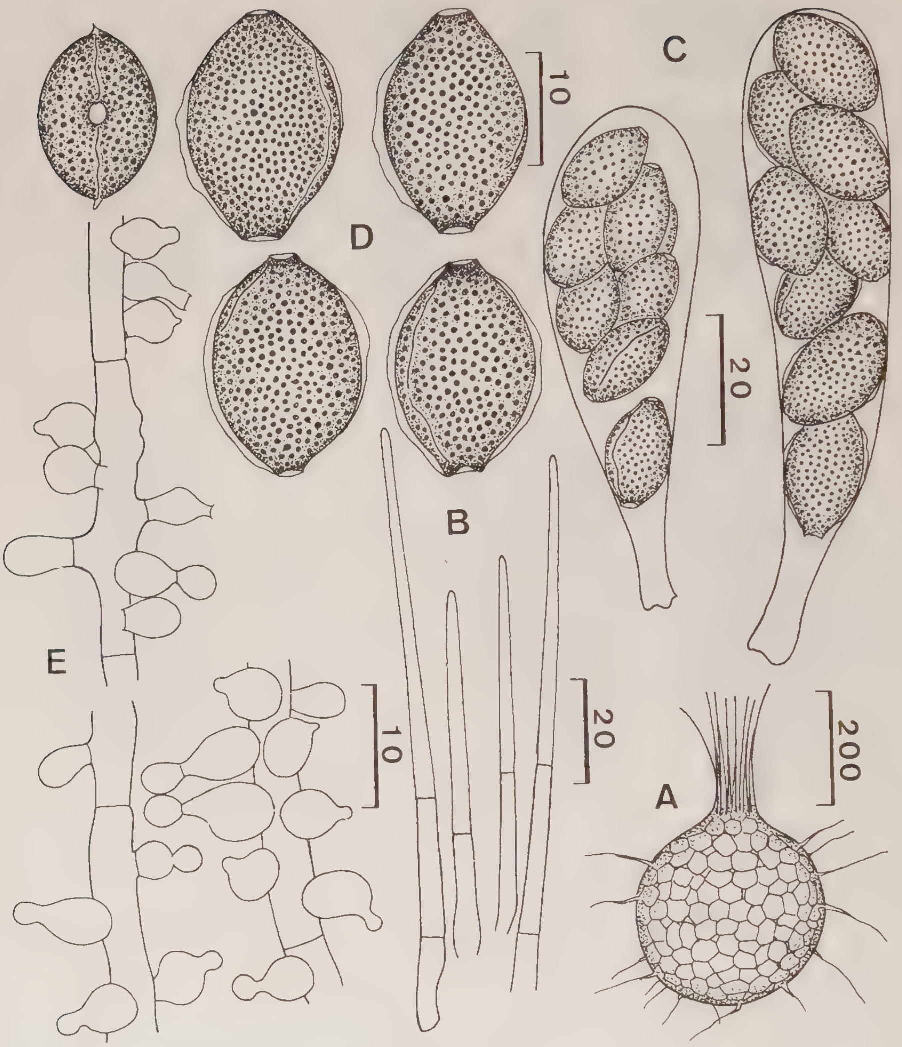
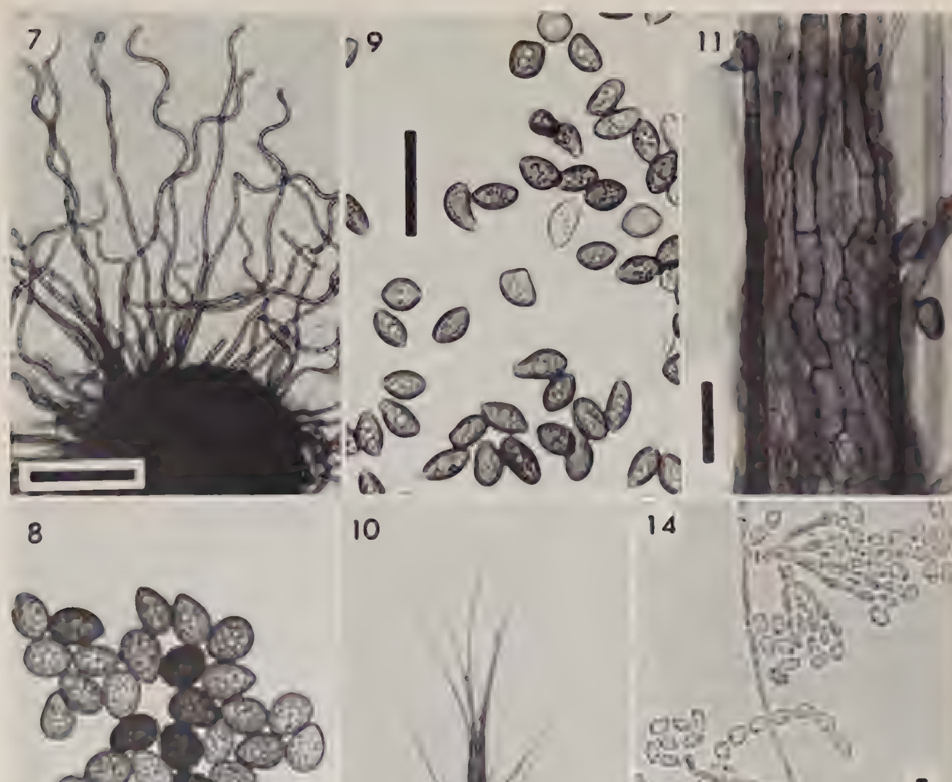


Fig. 6. *Pteridiosperma lenticularis*, NHL 2880. A. Perithecium. B. Ostiolar hairs. C. Asci. D. Ascospores (one in top view). E. Conidia. (All arabic numerals in scales denote μm.)

ab fronte latiore visis, (17–)20–22(–24) × 12–14 × 10–12 μm, primum hyalinis, deinde profunde olivaceo-brunneis vel atribrunneis, opacis, utrinque truncatis, in parte convexa foveolatis, cristis angustis demissis, 1.0–2.0 μm crassis, sinuosis, aliformibus ornatis, ab utroque termino poro germinali circa 2.0 μm diam praeditis.

Mycelio e hyphis hyalinis vel dilute stramineis, ramosis, septatis, 2.5–6.5 μm diam composito. Conidiophoris nullis. Cellulis conidiogenis (monophialidibus) integris, terminalibus, determinatis, ampulliformibus, 4.0–12.5 × 2.5–6.5 μm, basi inflatis, ad apicem angustatis, dispersis in hypha. Conidiis unicellularibus, hyalinis, ovatis vel



Figs. 7–8. *Chaetomium amygdalisporum.* 7. Terminal hairs. 8. Ascospores.
Fig. 9. *Chaetomium citrinum.* Ascospores.
Figs. 10–12. *Farrowia malaysiensis*, NHL 2260. 10. Perithecium. 11. A portion of perithecial neck. 12. Ascospores.
Figs. 13–14. *Kernia retardata.* 13. Section of cleistothecium. 14. Conidia.
(Scales, 7, 10 = 100 μm; 8, 9, 11, 14 = 20 μm; 12 = 10 μm; 13 = 50 μm.)

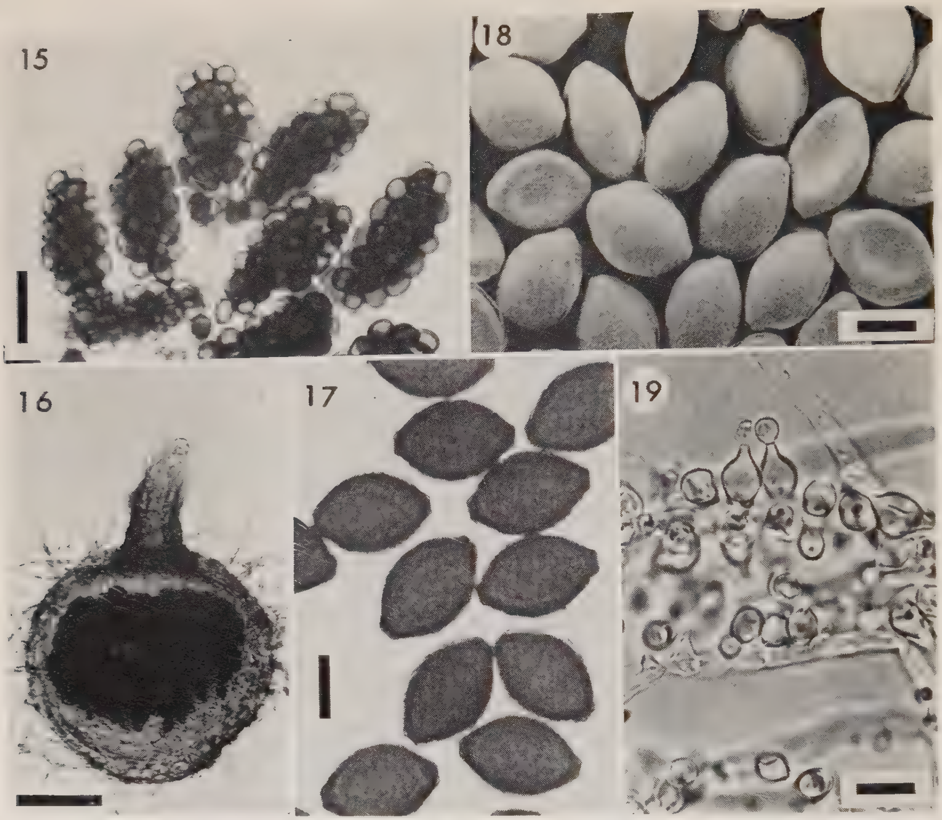


Fig. 15. *Preussia fleischhakii*. Asci.
Figs. 16–19. *Pteridiosperma lenticularis*. 16. Perithecium. 17. Ascospores. 18. SEM micrograph of ascospores. 19. Phialides and conidia. (Scales, 15 = 20 μm; 16 = 100 μm; 17, 18, 19 = 10 μm.)

pyriformibus, (3.0–)4.0–5.0 × 2.5–4.0 μm, basi truncatis vel plus minusve acutis, superne rotundatis, levibus, saepe in globis viscosis aggregatis.

Coloniis in agaro 'potato-carrot' ad 23 C conspicue restrictis; peritheciis et conidiis vix visis.

Holotypus—in vaccarum fimo, Nishinasuno-machi, Nasu-gun, Tochigi, in Japonia, 8.x.1978, legit. T. Muroi, No. 2880. In Herbario NHL.

Etymology: *lenticularis* = lens-shaped, referring to ascospore shape.

Krug and Jeng (1979) erected the genus *Pteridiosperma* for an atypical pair of *Microthecium* species, *M. foveolatum* Udagawa & Horie (Hawksworth and Udagawa, 1977) and *M. ciliatum* Udagawa & Takada (1974), which have superficial cleistothecia. They differ from most *Microthecium* species in their ascospores which have longitudinal, wing-like ridges that often anastomose to form an irregular reticulum. This third fungus is rather similar to *P. foveolata* (Udagawa & Horie) Krug & Jeng and differs in having perithecial ascocarps, asci which are twice as long, and oblate ascospores with two wing-like ridges instead of the three to four found in the latter species.

Chaetomium megasporum Sörgel in Seth, Beih. Nova Hedwigia **37**: 82. 1970 [1972].

≡*Achaetomiella megaspora* (Sörgel) D. Hawksw., Trans. Br. mycol. Soc. **65**: 136. 1975.

=*Achaetomiella fusispora* Calviello, Darwiniana **18**: 558. 1974.

=*Chaetomium piluliferoides* Udagawa & Horie, Trans. mycol. Soc. Japan **16**: 337. 1975.

In the fourteenth paper of this series, Udagawa and Horie (1975) proposed *C. piluliferoides* as the *Chaetomium* isolate from grassland soil, Sugadaira, Sanada-machi, Chiisagata-gun, Nagano, Oct. 17–19, 1972, NHL 2738. The isolate has strongly fusiform ascospores, which are reminiscent of those of *C. megasporum* Sörgel (Seth, 1970) and *Achaetomiella fusispora* Calviello (1974), though no anamorph of the latter two species has ever been reported. After examining the appropriate types, Hawksworth (1975b) concluded that *A. fusispora* was synonymous with *C. megasporum* which was at one time included within *Achaetomiella*. Recently Udagawa (1980) has considered *Achaetomiella* synonymous with *Chaetomium*, because distinctions between the two genera were not sufficient to warrant separation. Moreover, it was found that the Japanese isolate described as *C. piluliferoides* is entirely identical with *C. megasporum* and thus in this paper *C. piluliferoides* is considered synonymous with it. It was also found that there was a *Botryotrichum* anamorph in the IFO 30793 strain of *C. megasporum*.

We express with pleasure our gratitude to Dr. John C. Krug, University of Toronto, for his helpful suggestions concerning *Pteridiosperma*. We wish also to thank the curators of the ATCC, CMI and IFO, for supplying the standard cultures used in this study.

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摘　　要

日本産子のう菌類として次の7種を記載した.

Apiosordaria microcarpa sp. nov.（ソルダリア科）富山県の水田土壌から分離，*Chaetomium amygdalisporum* sp. nov.（ケトミウム科）群馬県の土壌から分離，*Chaetomium citrinum* sp. nov.（ケトミウム科）および *Kernia retardata* sp. nov.（ミクロアスクス科）栃木県の水田土壌から分離，*Pteridiosperma lenticularis* sp. nov.（メラノスポラ科）栃木県の牛糞から分離，*Farrowia malaysiensis* D. Hawksw.（ケトミウム科）鹿児島県の土壌から分離，*Preussia fleischhakii* (Auersw.) Cain（スポロールミア科）和歌山県の河川底泥から分離.

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Trans. mycol. Soc. Japan (日菌報) 22: 27–36, 1981

Notes on the Japanese rust fungi VII *Peridermium yamabense* sp. nov., a pine-to-pine stem rust of white pines

Haruyoshi SAHO*

佐　保　春　芳*：日本産銹菌ノート Ⅶ　五葉松直接感染型幹銹菌
Peridermium yamabense sp. nov.

Summary

Aeciospores of a rust on twigs of *Pinus pumila* resembling *Cronartium ribicola* were inoculated on white pines and hard pines. Positive results were obtained only on white pines. After inoculation, aeciospores were produced on *Pinus pumila*, *P. strobus*, *P. monticola* and *P. strobiformis*. On *P. griffithii*, *P. peuce* and *P. pentaphylla*, definite fusiform swellings and discoloration were found. Because rust hyphae were recognized in the host cells, those pines seemed to be susceptible to the rust. Therefore, all tested white pines were positive to the rust, while *P. densiflora* and *P. thunbergii*, hard pines, were negative. The causal fungus is discussed as a new species, *Peridermium yamabense* Saho et I. Takahashi, the first known pine-to-pine stem rust of white pines.

In 1972 a caulicolous rust resembling *Cronartium ribicola* was found on twigs of *Pinus pumila* Regel at the Tokyo University Forest in Hokkaido (Figs. 1, 2). Since then studies of cytology of germ tubes, anatomical observation of rusted swellings and relationship between nucleus of host cells and penetrating hyphae of the rust have been reported (Saho, 1974a, 1974b, 1978; Saho and Takahashi, 1973, 1976a, 1976b). Inoculation experiments on putative alternate host plants, *Ribes* and *Pedicularis*, were made, but no positive results were obtained (Saho and Takahashi, 1976b). Results of those experiments indicated that this rust had the same monokaryotic condition of germ tubes as recorded in previous reports for the pine-to-pine stem rust of hard pines (Christenson, 1968; Hiratsuka, 1968, 1969, 1973a, 1973b; Hiratsuka and Maruyama, 1968; Hiratsuka, et al., 1966; Hiratsuka and Powell, 1976; Krebill and Nelson, 1970; Zalasky and Riley, 1963). Therefore, it was postulated that this rust is a pine-to-pine stem rust of white pines in the genus *Peridermium* (Saho and Takahashi, 1976b).

However, spraying aeciospores of this white pine rust directly on needles and stems failed to produce infection. The same was true for Olembo's (1971) needle inoculation with *Peridermium pini* on *P. sylvestris*. This rust has long been known to infect directly pine to pine, especially through stem wounds. So inoculations through wounds with

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the white pine rust were carried out from 1976 to 1979. These experiments, and the causal fungus as a new species are discussed here.

Inoculations on *P. pumila*, *Ribes* and *Pedicularis* were made by Mr. Takahashi at the Tokyo University Forest in Hokkaido. Inoculations on foreign pines, *Ribes* and *Pedicularis*, cytological and anatomical studies, and scanning electron microscopy were made by the author.

Methods of inoculation and materials

Because no clear results had been obtained by spraying spores, two other methods were tried on white pines and hard pines.

Knife method: A wound was made diagonally by knife in the bark, and spores collected from aecial blisters on *P. pumila* were applied to the wound. Then the curved bark was pressed back on the wound and was covered by plastic tape.

Injection method: Spores suspended in water were injected directly into pine shoots by syringe. This method was the best for treatment of many seedlings in a limited time, and was applied to *Peridermium pini* by Klingström (1972).

The inoculated pines were the white pines *Pinus pumila* Regel, *P. pentaphylla* Mayr (both native to Japan), *P. strobus* L., *P. monticola* Dougl., *P. peuce* Grieseb., *P. griffithii* McClel. and *P. strobiformis* Engelm., and the hard pines *P. densiflora* Sieb. et Zucc. and *P. thunbergii* Parl. (both native to Japan). Their ages varied from three to fifteen years, except *P. pumila* growing at the mountain top. Young seedlings were in pots and older ones were in the arboretum at the Tohoku Branch, Forestry and Forest Products Research Institute. All seedlings of foreign species were obtained from seeds which were exchanged under the co-operation of IUFRO (International Union of Forest Research Organization) Division 2, Working Party S.2.03.02 'Breeding white pines'.

Results of inoculation experiments

Knife method: On *P. pumila*, potted three-year-old seedlings were inoculated with spores on June 11, 1976. The inoculations were conducted at the mountain top, Mt. Dairoku, 1,450 m above sea level in the Tokyo University Forest in Hokkaido. Spores were collected from aecial blisters and were put under the bark of seedlings as

Figs. 1–2. Naturally infected *Pinus pumila* collected at the Tokyo University Forest in Hokkaido. Top of shoot is affected. No aecial blisters were found on older main stems. Figs. 3–4. Aecial blisters on artificially inoculated *P. pumila*. (pictures taken by Mr. I. Takahashi). Fig. 5. Inoculated *P. strobus* by knife method on two-year-old shoot. Aecial blisters appeared around and below portion of inoculated point. Fig. 6. Inoculated *P. strobiformis* by injection method on a current shoot. Aecial blisters on swollen spurs. Fig. 7. Inoculated *P. monticola* by injection method. Fig. 8. Inoculated *P. griffithii*. After inoculation on two-year-old twig in 1977, swelling appeared in 1979, but no aecial blisters have not been found up to 1980.



Figs. 1–8.

soon as possible after collection. Swellings of inoculated portions were found beginning of late May, 1978. The faster growth of the fungus was towards the larger branch, because infections appeared to spread with discoloration below the infected points much more rapidly than above them. Aecial blisters first appeared in June of the same year (Figs. 3, 4).

At the same time inoculations on naturally growing *P. pumila* were made. In this case it was difficult to determine whether aecial blisters originating from natural infection or artificial inoculation. Becuase they were placed at the mountain top and were difficult to observe through winter, precise changes on discoloration were not recorded.

On *P. strobus*, three- to five-year-old seedlings were inoculated on June 25, 1978 at the Tohoku Branch, Forestry and Forest Products Research Institute, Morioka. One month later, discoloration of bark appeared starting around the inoculated portion and expanding rapidly along the shoot. In summer of the next year, much wider spread of discoloration on bark below infected points was observed than above, and wilting of needles above the inoculated point was recognized. In late summer, these needles were dead, while needles on the shoot below the inoculated point were healthy. Anatomical observation of the inoculated shoot showed that rust hyphae penetrated the host cells. On May 20, 1980, aecial blisters were found on some shoots, but most of the inoculated shoots were killed by the rust. When inoculation was made on two- to three-year-old stems, aecial blisters were produced two to three years after inoculation (Fig. 5).

Additional inoculation experiments were made on June 20, 1979. The inoculated pine species were *P. strobus*, *P. strobiformis*, *P. peuce*, *P. monticola*, *P. pentaphylla* and *P. koraiensis*. Results are expected in 1981 or 1982.

Injection method: Injection with spore suspension was made directly into seedlings and young trees at the Tohoku Branch. Spores were collected at the University Forest in Hokkaido and were sent to the Tohoku Branch, where they were stored in a refrigerator.

On *P. pumila*, inoculation experiments were made on June 20, July 17, 1977 and June 20, 1978. Seedlings of *P. pumila*, three- to five-year old, were so small that discoloration was hard to observe, but swellings were found. In the next year, most of inoculated seedlings were killed by the rust before production of aecial blisters. Even when only one twig among several was inoculated, whole four- to five-year old plants were killed. The Tohoku Branch is located at lower altitude than Mt. Dairoku and it becomes very hot in summer. This high temperature might weaken the inoculated seedlings and causes their death.

At the University Forest, injection of spores was conducted on twigs of naturally growing *P. pumila*, too. New production of aecial blisters was observed but it was not clear whether from natural or artificial infections. Because of difficulty in making continuous observations through winter at the mountain top, no precise observation was recorded on inoculated pines.

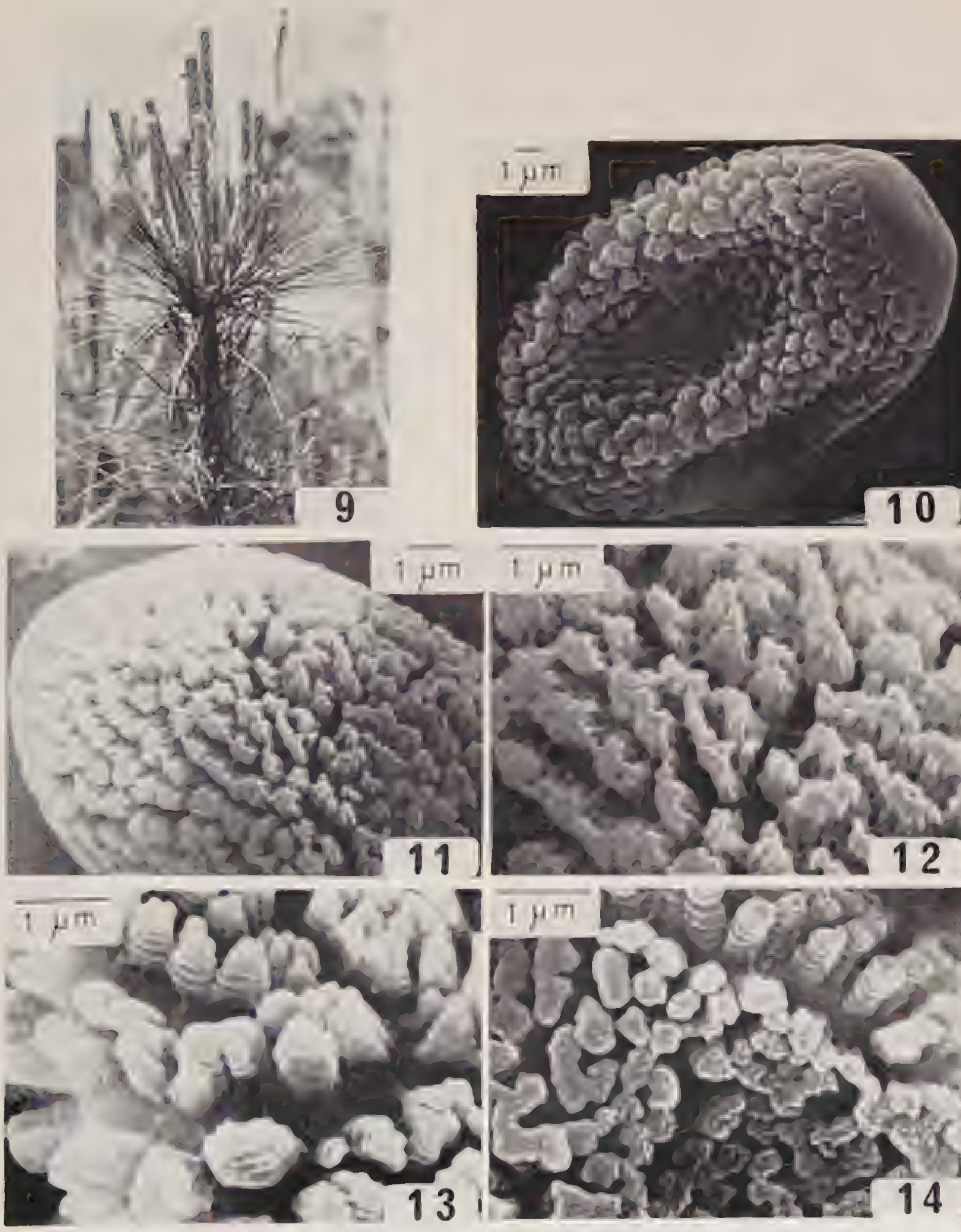


Fig. 9. Fifteen current shoots on *P. strobus* are produced on a node just above the inoculated point. Figs. 10–12. Aeciospores of *Peridermium yamabense* by SEM. Fig. 13. Warts on aeciospores of *Peridermium harknessii* collceted in Canada by SEM. Fig. 14. Warts on aeciospores of *Peridermium pini* collected in Scotland by SEM.

On *P. strobus*, inoculated one-year-old shoots showed resinosis and stopped their growth in diameter. Many small shoots commonly appeared at a node just above the inoculated portion in the next year and needles on those shoots became brown. At those nodes up to 15 shoots appeared (Fig. 9), contrasted with five to six shoots at a node in healthy trees. By anatomical observation, rust hyphae penetrated into cells of shoots, just as in the knife method. One year after inoculation, some shoots around the inoculated points were dead. Therefore, one-year-old shoots of *P. strobus* seemed to be too susceptible to produce aecial blisters. However, Bolland (1957) mentioned that aecia of *Peridermium pini* produced on current shoots on *P. sylvestris* through wounds and older twigs showed resistance to the rust. In case of the white pine rust, current shoots of *P. strobus* were too susceptible, and when two- to three-year-old twigs were inoculated, aecial blisters were produced two to three years later.

On *P. strobiformis*, after inoculation on June 20, 1978, aeciospores were obtained on May 28, 1980. In this case spores were injected into current shoots (Fig. 6).

On *P. monticola*, inoculations were made on June 20, 1977 and aeciospores were collected on May 22, 1979. Those aeciospores were produced on a four-year-old twig inoculated at the age of two (Fig. 7).

On *P. griffithii*, inoculated on June 20, 1977, definite swellings appeared and the rust hyphae were observed in the cells of the swellings. But no aeciospores had appeared by 1980 (Fig. 8).

On *P. peuce* and *P. pentaphylla* inoculated on June 20, 1977 and June 19, 1978, discoloration and resinosis were recognized, but no aeciospores were seen up to 1980. These two pine species were adjudged susceptible to the rust, since the rust hyphae were found in the host cells.

On the contrary, *Pinus densiflora* and *P. thunbergii*, hard pines, showed negative results with no swelling and no discoloration after inoculation. By the anatomical observation, no penetrating hyphae of the rust into the host cells were observed. Therefore, these two pines were considered immune.

Inoculation experiments with aeciospores on putative alternate host plants.

Because no positive results were obtained on *Ribes rubrum* L., *R. grossularia* L., *R. sachalinense* Nakai, *Pedicularis chamissonis* Steven var. *japonica* (Miq.) Max. and *P. yezoensis* Max. (Saho and Takahashi, 1976b), further inoculation experiments with aeciospores were made on June 20, 1978 on potted plants of *Pedicularis chamissonis* var. *japonica*, *P. oederi* Vahl and *P. resupinata* L. at the University Forest in Hokkaido and also at the Tohoku Branch. After inoculation, plants were kept in a moist chamber for ten to fifteen days. As before, no uredosori were produced. For these consistently negative results, it can be stated that this rust infects pine directly and has no alternate host plants.

Observation by scanning electron microscopy

Aeciospores of the rust on *P. pumila*, pine-to-pine stem rust of hard pines (*Peridermium pini* and *P. harknessii*) and heteroecious rusts in the genus *Cronartium* were examined by a scanning electron microscope. The results showed that ornamentation of warts of the white pine *Peridermium* has irregular and vertical streaks (Figs. 10, 11, 12). On the other hand, ornamentation of *P. pini* and *P. harknessii* showed horizontal streaks like piled-up dishes (Figs. 13, 14). Ornamentation of *Cronartium ribicola*, *C. coleosporioides* and *C. comptoniae* also showed horizontal streaks (Hiratsuka, 1970; Hiratsuka and Powell, 1976). Only *C. comandrae* showed ornamentation somewhat similar to that of white pine *Peridermium*, but it has pyriform aeciospores (Hiratsuka and Powell, 1976). Japanese specimens of *C. ribicola* [=*C. kamtschaticum*, alternate host plant is *Pedicularis*] (Yokota and Uozumi, 1976) was also examined by the author. The result was the same as *C. ribicola* of the American and Canadian specimens having ornamentation of horizontal streaks.

From results of comparisons of spore ornamentation, the white pine *Peridermium* can be distinguishable mainly by a scanning electron microscope but also by a light microscope from other species in the genera *Peridermium* and *Cronartium* on pines.

Description of the rust

For the reasons described above, a rust on twigs of *Pinus pumila* is proposed as a new species in the genus *Peridermium*. This is the first known pine-to-pine stem rust of white pines.

Peridermium yamabense **Saho et I. Takahashi**, sp. nov.

Spermogonia in cortice immersa, indeterminata; sori aecidiis Cronartii similes, ab cortice erumpentes in tumulis ca. 2 mm diam; peridia saccata; sporae globosae vel ellipticae, aurantiacae, (22–) 24–30 (–33) × (16–) 17–20 (–21) μm; episporia verrucosa; verrucae verticaliter lineatae. Basidiosporae nullae.

Specimens examined: On stems and twigs of *Pinus pumila*; Mt. Dairoku, the Tokyo University Forest in Hokkaido, Furano, Hokkaido, June 6, 1972 (Holotype); June 13, 1973; June 8, 1974, June 17, 1975; June 3, 1976; June 17, 1977; June 13, 1978; July 5, 1979; June 30, 1980.

On twigs of *Pinus pumila*; June 20, 1978, inoculated on June 20, 1976 at the Tokyo University Forest in Hokkaido.

On twigs of *P. pumila*; Yukoman-betsu, Mt. Taisetsu, Hokkaido, TUFH-5352, July 14, 1973.

On *P. strobus*; May 2, 1980, inoculated on June 25, 1978.

On *P. strobiformis*; May 28, 1980, inoculated on June 20, 1978.

On *P. monticola*; May 22, 1979, inoculated on June 20, 1977.

These specimens from inoculations on foreign tree species were collected at the Tohoku Branch, Forestry and Forest Products Research Institute, Morioka, Iwate.

The type specimen and inoculated foreign tree specimens are deposited in the Saho herbarium of the Kansai Branch, Forestry and Forest Products Research Institute, Kyoto. The other specimens on *P. pumila* are deposited in the herbarium of the Tokyo University Forest in Hokkaido, Furano, Hokkaido. The name '*yamabense*' is based upon the name of the town 'Yamabe' where the main office of the University Forest is located.

Discussion and conclusion

Three species of pine-to-pine stem rust are parasitic on hard pines. They are *Peridermium pini* (=*Endocronartium pini*) in Europe, and *P. harknessii* (=*E. harknessii*) and *P. filamentosum* in the North America. Their life cycles were studied by Christenson (1968), Krebill and Nelson (1970), Klingström (1972), Olembo (1971) and Hiratsuka et al. (1966). For the pine-to-pine stem rust of hard pines, the genus *Endocronartium* was established based mainly upon cytology and morphology of germ tubes by Hiratsuka (1969). But with dried specimens, nobody can make germination experiments. If germination experiments are necessary to determine the genus *Endocronartium*, it seems undesirable for fungus taxonomy.

Peterson (1967) mentioned that *Peridermium pini* is the type species of *Peridermium*. But *Endocronartium* contains the type of *Peridermium*. Therefore, Laundon (1973, 1976) stated that *Endocronartium* is illegitimate. The author agrees with their opinions, so the new pine-to-pine stem rust of white pines is proposed in the genus *Peridermium*.

Endocronartium inflaticalcaratum, without Latin description, was used provisionally and tentatively for discussion of the new rust (Saho and Takahashi, 1976a). Then it was proposed in the genus *Peridermium* (Saho and Takahashi, 1976b). The informal specific epithet '*inflaticalcaratum*' is ambiguous and is not easy to pronounce. The author withdraws that specific epithet and formally proposes '*yamabense*' for the rust.

The author is deeply grateful to Dr. D. M. Henderson (The Royal Botanic Garden, Edinburgh, Scotland), Dr. H. B. Gjaerum (Statens Plantvern, Ås, Norway), Dr. G. F. Laundon (Plant Diagnostic Service, Levin, New Zealand) for helpful suggestions, and to Dr. Y. Hiratsuka (Northern Forest Research Centre, Edmonton, Canada), Dr. F. Roll-Hansen (Norwegian Forest Research Institute, Ås, Norway) and Dr. J. S. Murray (University of Aberdeen, Scotland) for specimens of various rust species, and the late Mr. T. Uozumi (Hokkaido Branch, For. and For. Prod. Res. Inst.) for specimens of *Cronartium ribicola* in Hokkaido.

The author is indebted to Dr. R. S. Peterson (St. John's College, Santa Fe, New Mexico, U. S. A.) and Dr. J. S. Murray for reading manuscript. Many thanks due to Mr. T. Ogawa (Forest Regional Office, Obihiro, Hokkaido) for his assistance with seedlings of *Pedicularis resupinata* and to the members of the University Forest for their help with seedlings of *P. pumila*, and to Mr. Y. Abe (Mushroom Laboratory, For. and For. Prod. Res. Inst.) for his assistance with a scanning electron microscope.

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摘　　要

東京大学北海道演習林内の大麓山頂（標高 1450m）附近に生育しているハイマツに，五葉松発疹銹病菌と酷似する銹菌が発見された．銹胞子の発芽実験などを経て，この銹菌はマツからマツへ直接感染する菌と推定された．その後，接種試験によって各種の五葉松類にのみ感染することを明らかにした．これに対し，二葉松類への接種試験は不成功に終った．さらに形態的には，特に銹胞子のいぼの形状に特徴があり，他の二葉松直接感染型銹菌や五葉松発疹銹病菌とは全く異ることが明らかとなった．これらの理由から，この銹菌を *Peridermium yamabense* Saho et I. Takahashi, sp. nov. として報告した．

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Notes on some plant-inhabiting Ascomycotina from western Japan (2)*

Ken KATUMOTO**

勝 本 謙**：西日本産植物寄生子囊菌類知見 (2)*

Summary

Four new species of the Pyrenomycetes, *Hyponectria dicalycis* Katumoto and *Leiosphaerella dicalycis* Katumoto on *Dicalyx lucida*, *Leiosphaerella ilicis* Katumoto on *Ilex pedunculosa* and *Dicarpella rubicola* Katumoto on *Rubus buergeri*, are described. Morphological aspects of *Phyllachora ficuum* Niessl and *P. fici-wightianae* Sawada, and the occurrence of *Mamiania fimbriata* (Pers. ex Fr.) Ces. et de Not. in western Japan are also noted.

In this paper records of seven Pyrenomycetes from western Japan are presented. Two species of *Leiosphaerella* Höhn. in the Amphisphaeriaceae and each one species of *Hyponectria* Sacc. in the Physosporellaceae and *Dicarpella* H. et P. Syd. in the Pseudovalsaceae are described as new ones. Two *Phyllachora* species in the Melogrammataceae and *Mamiania fimbriata* in the Diaporthaceae are also noted. All the specimens studied in this paper are deposited in the Herbarium of the Faculty of Agriculture, Yamaguchi University (YAM).

7. ***Phyllachora ficuum*** Niessl, Hedwigia **20**: 99. 1881. (Figs. 3, 4)

≡ *Trabutia ficuum* (Niessl) Theiss. et H. Syd., Ann. Mycol. **13**: 352. 1915.
= *Auerswaldia microthyrioides* P. Henn., Hedwigia **43**: 142. 1904.
≡ *Trabutiella microthyrioides* (P. Henn.) Theiss. et H. Syd., Ann. Mycol. **13**: 359. 1915.
= *Catacauma nipponicum* H. et P. Syd., Ann. Mycol. **13**: 40. 1915.

See von Arx and Müller (1954) for additional synonyms.

Stromata mostly epiphyllous and subcuticular, sometimes hypophyllous and subepidermal, gregarious, orbicular to somewhat irregular in shape, 0.3–1.0 mm in diam. with few perithecia; asci clavate, 40–75 × 14–20 μm; ascospores biseriate, elliptic, 12–15 × 8–10 μm.

Specimens examined: On the living leaves of *Ficus erecta* Thunb. (*Inubiwa*). Shizukiyama, Hagi-shi, Yamaguchi Pref. (Oct. 16, 1950. I. Hino-YAM 20637); Shiroyama,

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* (1): Trans. mycol. Soc. Japan **21**: 7–16, 1980.

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Kagoshima-shi, Kagoshima Pref. (Dec. 6, 1952. Y. Ubemoto-YAM 20638); Kanda-mura, Toyora-gun, Yamaguchi Pref. (Dec. 7, 1952. I. Hino-YAM 20636); Shuho-cho, Mine-gun, Yamaguchi Pref. (July 31, 1962. K. Katumoto-YAM 20639); Kasayama, Hagi-shi, Yamaguchi Pref. (Nov. 2, 1969. K. Katumoto-YAM 23532); Tano, Miyazaki-shi, Miyazaki Pref. (Oct. 25, 1979. Y. Harada-YAM 23412). On the living leaves of *Ficus erecta* var. *sieboldi* King (*Hosoba-inubiwa*). Kurifu, Yaku Isl., Kagoshima Pref. (Dec. 24, 1971. K. Katumoto-YAM 22039).

Distribution: Tropics to warmer regions of Asia and Africa.

This fungus is very common on the leaves of *Ficus erecta* in western Japan, and has been known as *Trabutiella microthyrioides*, the type species of the genus *Trabutiella* Theiss. et H. Syd. *Trabutiella microthyrioides* was mentioned by von Arx and Müller (1954) to be a subcuticular form of *Phyllachora ficuum*. Another fungus, *Catacauma nipponicum* H. et P. Syd., has been known on the same host plant in Japan and was said to form its stromata in epidermal and parenchymatous cells of the lower side of leaves. However, *T. microthyrioides* and *C. nipponicum* sometimes occur on the upper and lower sides, respectively, of the same leaf-blade of *Ficus erecta*, and these are morphologically indistinguishable from *P. ficuum*. Therefore, *C. nipponicum* is here considered to be synonymous with *P. ficuum*.

8. ***Phyllachora fici-wightianae*** Sawada, Trans. Nat. Hist. Soc. Formosa **24**: 77. 1916. (Figs. 5, 6)

Stromata epiphyllous, subcuticular, indefinitely effuse, gregarious, raised and thick, black, shining, uneven with slightly protruded ostiolar portions of perithecia, 1–10 mm in diam; stromatal wall mostly plectenchymatous with longitudinally striated cells, at the upper portion clypeus-like and pseudoparenchymatous; locules globose to somewhat depressed globose, abundant in a stroma, 250–400 μm in diam; ascospores oblong, continuous, hyaline, smooth, $12–15 \times 6.5–7.5$ μm.

Specimen examined: On the living leaves of *Ficus superba* Miq. var. *japonica* Miq. (*Ako*). Experimental Forest of Ryukyu University, Yona, Okinawa Isl., Okinawa Pref. (Aug. 11, 1965. I. Hino-YAM 23526).

Other specimen examined: On *F. superba* var. *japonica*. Taipei, Taiwan (June 6, 1926. S. Suzuki-YAM 20452).

Distribution: Taiwan and Japan (Kyushu).

This species somewhat resembles *P. ficuum*, but it is distinguishable from the latter species by indefinitely effused stromata and oblong ascospores. It has been known in Taipei of Taiwan (Sawada, 1919) and Yaku Island of Kyushu (Katsuki, 1953). Okinawa Island forms a connecting link between the former two habitats.

Sawada (1916) noted the presence of asci containing numerous secondary spores among normally developed asci with eight ascospores. The present author could not find out in his specimens any ascus with secondary spores as described by Sawada.

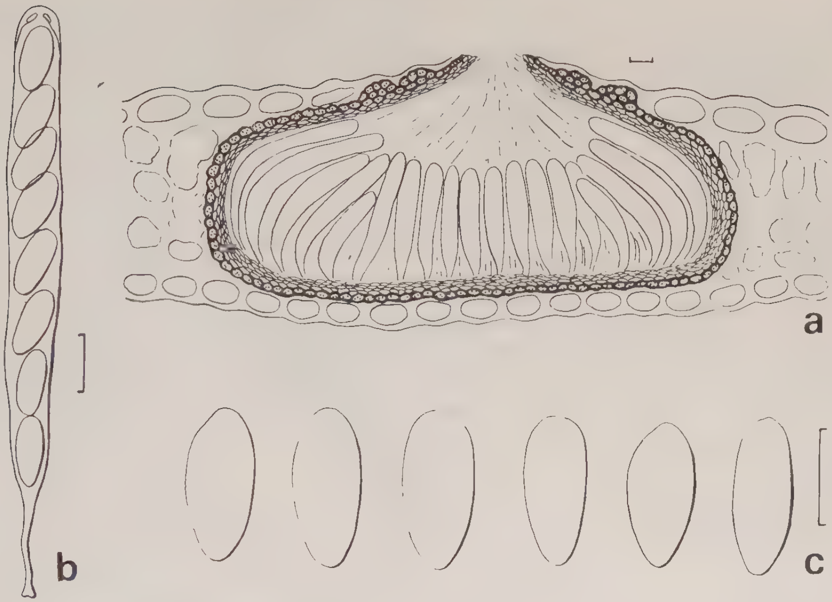


Fig. 1. *Hyponectria dicalycis*: a, Perithecium. b, Ascus. c, Ascospores. (Scales: 10 μm)

9. ***Hyponectria dicalycis*** Katumoto, sp. nov. (Figs. 1, 7, 8)

Peritheciis hypophyllis, subgregariis, subcutaneis, depresso-globosis vel interdum irregulariter lenticularibus, 200–260 μm latis, 80–140 μm altis; parietibus membranaceis, atro-brunneis, pseudoparenchymaticis, 9–14 μm crassis, ex cellulis exterioribus polyhedricis 5–8 μm diam et interioribus planis 6–10 $\times$ 1.5–2 μm compositis; ascis cylindraceis, unitunicatis, apice rotundatis, breviter stipitatis, octosporis, 80–100 $\times$ 5–7 μm; ascosporis unistichis, oblongis, continuis, apice utrinque obtusis, deorsum plus minusve attenuatis, hyalinis, laevibus, 10–14 $\times$ 3.5–5 μm.

Hab. in foliis vivis *Dicalycis lucidae* Hara (*Kuroki*). Hikoshima, Shimonoseki-shi, Yamaguchi Pref. (Sept. 6, 1960. I. Hino-YAM 20488: Holotypus); Yamaguchi-shi, Yamaguchi Pref. (Feb. 14, 1975. K. Katumoto-YAM 23534).

Without lesion; perithecia frequently showing the clypeal stromatic development in several epidermal cells around a very short papillate or almost plane ostiole, sometimes visible as dark circular spots around whitish centers; apical annulus of asci shallow, not stained blue with Melzer's reagent; paraphyses deliquescent at maturity.

The present fungus seems to have the closest relation to the fungi of the genus *Anisostomula* Höhn. in consideration of morphological aspects of perithecial wall and non-amyloid ascus. *Anisostomula* was comprised by Petrak (1923) in his family Hyponectriaceae with *Hyponectria* Sacc. which was originally erected in the Nectriaceae referring to the feature of perithecial wall. Von Arx and Müller (1954) and Müller and von Arx (1973) accepted the genus *Anisostomula* along with *Hyponectria* in the Polystigmataceae or

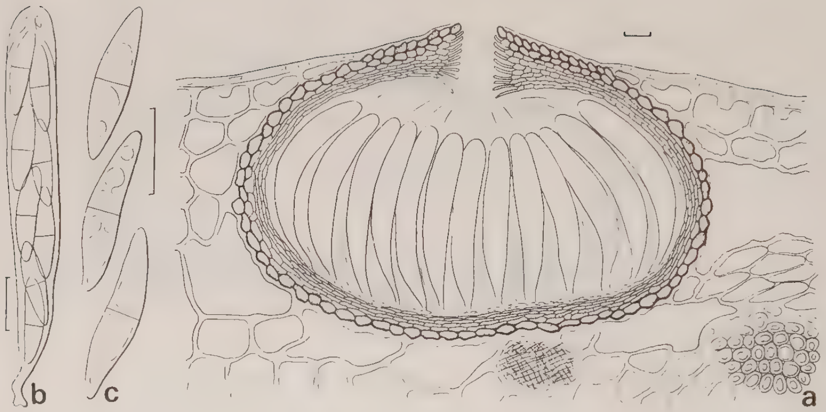


Fig. 2. *Leiosphaerella dicalycis*: a, Perithecium. b, Ascus. c, Ascospores. (Scales: 10 μm)

the Amphisphaeriaceae, respectively. *Anisostomula* has enlarged cells outside the perithecial wall, while *Hyponectria* has a perithecial wall composed of bright and flattened cells entirely. Barr (1976, 1977), however, treated *Anisostomula* as a synonym of *Hyponectria* which was placed by her in the Physosporellaceae Höhn. emend. Barr, an earlier name of the Hyponectriaceae, of the Diaporthales.

Nine species of *Hyponectria* were recognized by Barr (1977) in North America, and the author's fungus differs from these species in the morphological aspects of ascospores as well as in the host range. It is also distinguishable from members of the genus *Physalospora* Niessl, another genus having one-celled ascospores in the Physosporellaceae, by the plane ostiolar portion and the thin ascospore wall.

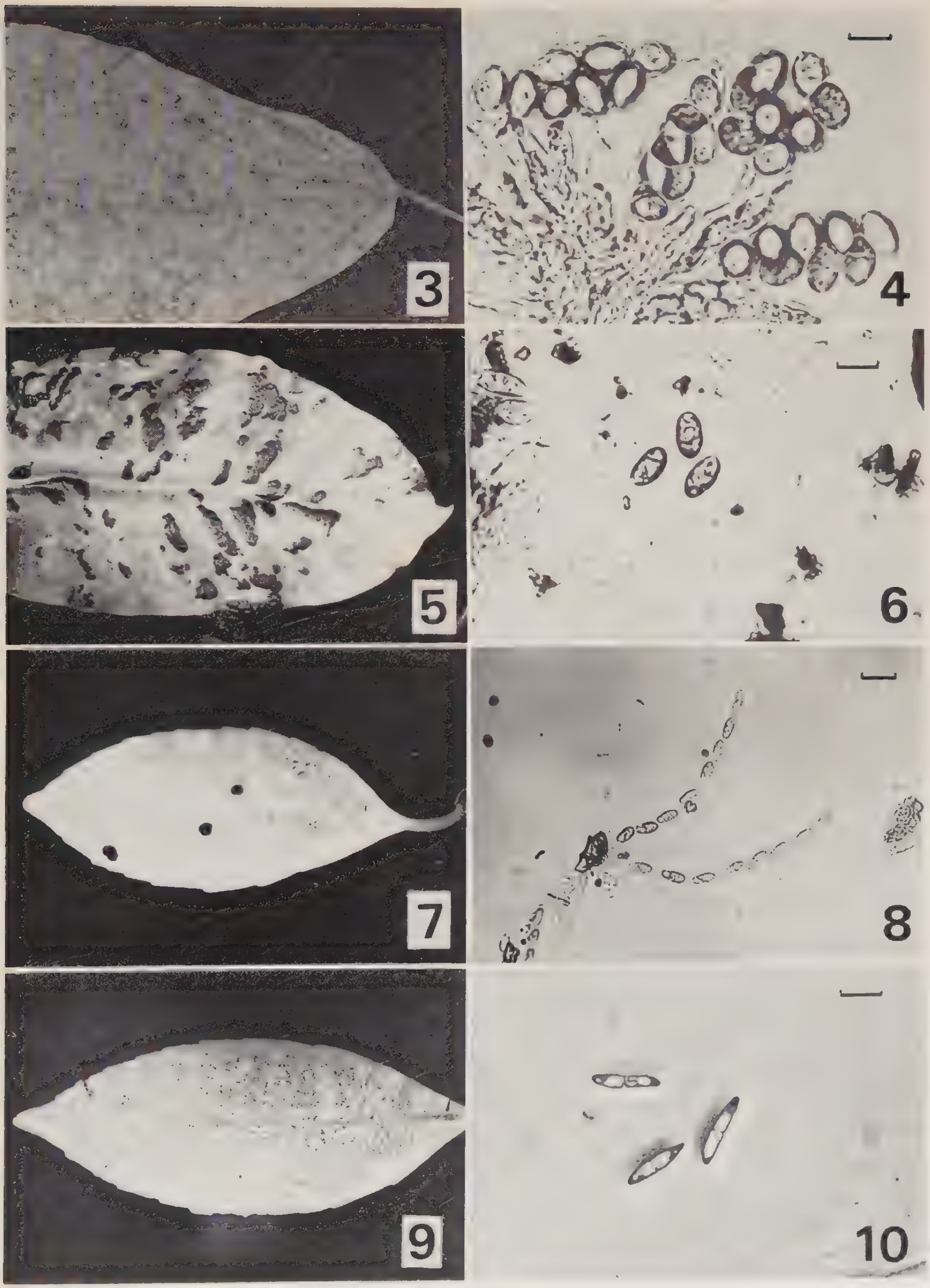
10. ***Leiosphaerella dicalycis*** Katumoto, sp. nov. (Figs. 2, 9, 10)

Peritheciis hypophyllis, subcutaneis, solitariis vel subgregariis, depresso-globosis, 170–200 μm latis, 80–130 μm altis, parietibus membranaceis, pseudoparenchymaticis, atro-brunneis, 12–17 μm crassis, ex cellulis polyhedricis 4–7 μm diam compositis; ostiolis rotundatis, planis, 20-25 μm diam; ascis cylindro-clavatis, unitunicatis, apice rotundatis vel leniter truncatis, breviter stipitatis, octosporis, $60–75 \times 7–8$ μm; ascosporis distichis, fusoideis, in medio 1-septatis, non vel vix constrictis ad septum, apice utrinque obtusis, plerumque leniter curvatis, hyalinis, laevibus, $16–20 \times 3–4$ μm.

Hab. in foliis vivis *Dicalycis lucidae* Hara (*Kuroki*). Yamaguchi-shi, Yamaguchi Pref. (Jan. 29, 1975. K. Katumoto-YAM 22629: Holotypus).

Without any lesion; apical annulus of asci distinctively stained blue with Melzer's reagent.

The present fungus apparently belongs to the genus *Leiosphaerella* Höhn. of the Amphisphaeriaceae referring to the amyloid apical annulus of asci and 2-celled hyaline ascospores. This fungus resembles *L. hyperborea* (Karst.) E. Müller, but it is distinguish-



Figs. 3–4. *Phyllachora ficuum*: 3, Stromata. 4, Asci and ascospores.
Figs. 5–6. *Phyllachora fici-wightianae*: 5, Stromata. 6, Ascospores.
Figs. 7–8. *Hyponectria dicalycis*: 7, Perithecia. 8, Asci.
Figs. 9–10. *Leiosphaerella dicalycis*: 9, Perithecia. 10, Ascospores (phase contrast micrograph).
(Scales: 4, 6 and 10, 10 μm; 8, 20 μm)

able from the latter species by somewhat slender ascospores. No species of *Leiosphaerella* has been known on plants of the Symplocaceae.

11. ***Leiosphaerella ilicis*** Katumoto, sp. nov. (Figs. 11, 13, 14)

Peritheciis epiphyllis, subcutaneis, solitariis, globosis, 120–140 μm diam; parietibus pseudoparenchymaticis, membranaceis, atro-brunneis, 10–15 μm crassis, apice leniter incrassatis, ex cellulis plane polyhedricis 5–10 × 2–3 μm compositis; ostiolis rotundatis, planis, 15–20 μm diam; ascis obclavatis, apice rotundatis, breviter stipitatis, octosporis, 65–80 × 10–14 μm; ascosporis distichis, fusoideis, in medio 1-septatis, ad septum vix constrictis, apice utrinque obtusis, hyalinis, 18–28 × 4.5–6 μm.

Hab. in foliis vivis *Ilicis pedunculosae* Miq. (*Soyogo*). Yoshida, Yamaguchi-shi, Yamaguchi Pref. (Dec. 10, 1975. K. Katumoto-YAM 23514: Holotypus); Hirakawa, Yamaguchi-shi, Yamaguchi Pref. (Dec. 13, 1980. K. Katumoto-YAM 23536).

Lesions circular, brownish to greyish brown, 4–8 mm in diam, with distinct margin which is reddish brown and slightly ridgy; apical annulus of asci distinctively stained blue with Melzer's reagent.

This fungus also belongs to the genus *Leiosphaerella*, and is distinguishable from *L. hyperborea* by larger ascospores, and from *L. praeclara* (Rehm) Höhn. by smaller ascospores. No species of *Leiosphaerella* has been recorded on plants of *Ilex*.

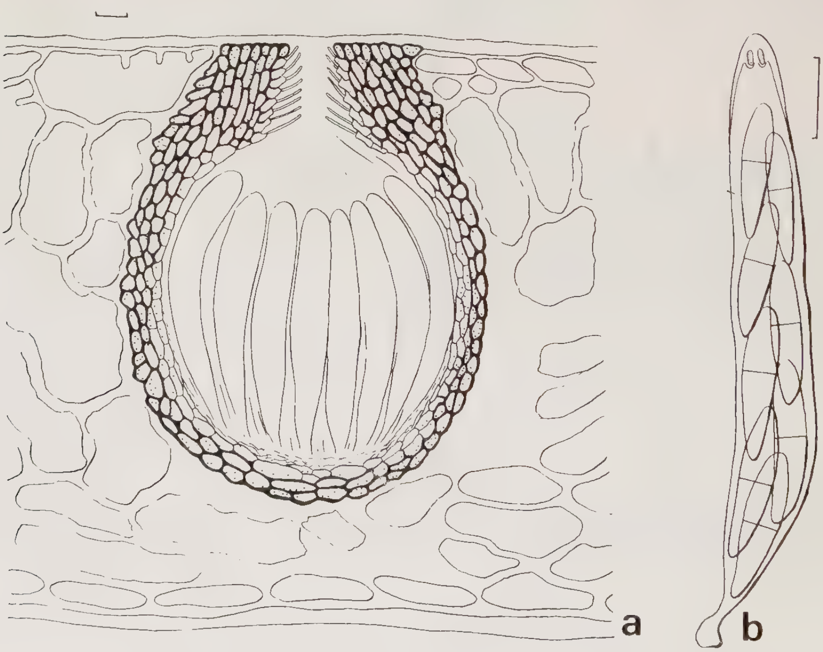


Fig. 11. *Leiosphaerella ilicis*: a, Perithecium. b, Ascus. (Scales: 10 μm)

12. ***Dicarpella rubicola*** Katumoto, sp. nov. (Figs. 12, 15–18)

Peritheciis epiphyllis, gregariis, subcutaneis, solitariis vel interdum conglutinatis, depresso-globosis, apice paulo pertuosis et ostiolatis, 150–170 μm latis, 80–110 μm altis; parietibus membranaceis, pseudoparenchymaticis, luteo-brunneis vel atro-brunneis, 9–14 μm crassis, ex cellulis exterioribus plane globosis 5–8 μm diam et interioribus lenticularibus 5–8 / 1.5–2 μm compositis; ascis clavatis, unitunicatis, apice rotundatis, breviter stipitatis, octosporis, 50–65 / 12–17 μm; ascosporis distichis, oblongo-fusoideis, plerumque deorsum leniter attenuatis, continuis, apice utrinque rotundatis, hyalinis, laevibus, 11–14 / 5–7 μm.

Hab. in foliis vivis *Rubi buergeri* Miq. (*Fuyu-ichigo*), Jifuku, Ato-cho, Abu-gun, Yamaguchi Pref. (Aug. 14, 1975, N. Miake-YAM 23533: Holotypus); Tenge, Yamaguchi-shi, Yamaguchi Pref. (Oct. 24, 1979, Y. Harada-YAM 23414); Karioto, Heki-cho, Ootsu-gun, Yamaguchi Pref. (June 22, 1980, K. Katumoto-YAM 23520).

Leaf spots circular to somewhat irregular, with indefinite margin, brownish, usually developing around an epiphyllous uredinium of *Cerotelium rubi* Rac., 6–15 mm in diam; perithecial wall thin and more or less flattened at the bottom, thick at the apex, frequently developing a stromatic pseudoparenchyma at the lateral portion; apical annulus of asci distinct, shallow, not stained blue with Melzer's reagent; paraphyses not observed.

The present fungus may find the most appropriate systematic position in the genus *Dicarpella* H. et P. Syd. in consideration of morphological features of perithecia, ascospores, and the non-amyloid apical annulus of asci. The genus *Dicarpella* was fully discussed by Barr (1978, 1979) and included into the Pseudovalsaceae of Diaporthales. The author's fungus is distinguishable from *D. bina* (Harkn.) H. et P. Syd. by narrower ascospores, and from *D. georgiana* (Miller et Thompson) Barr or *D. quercifolia* (Ell. et Ev.)

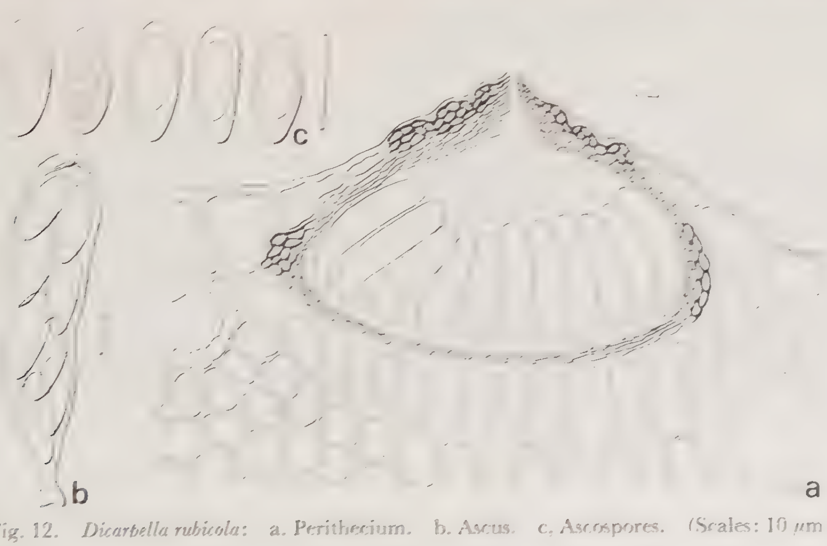
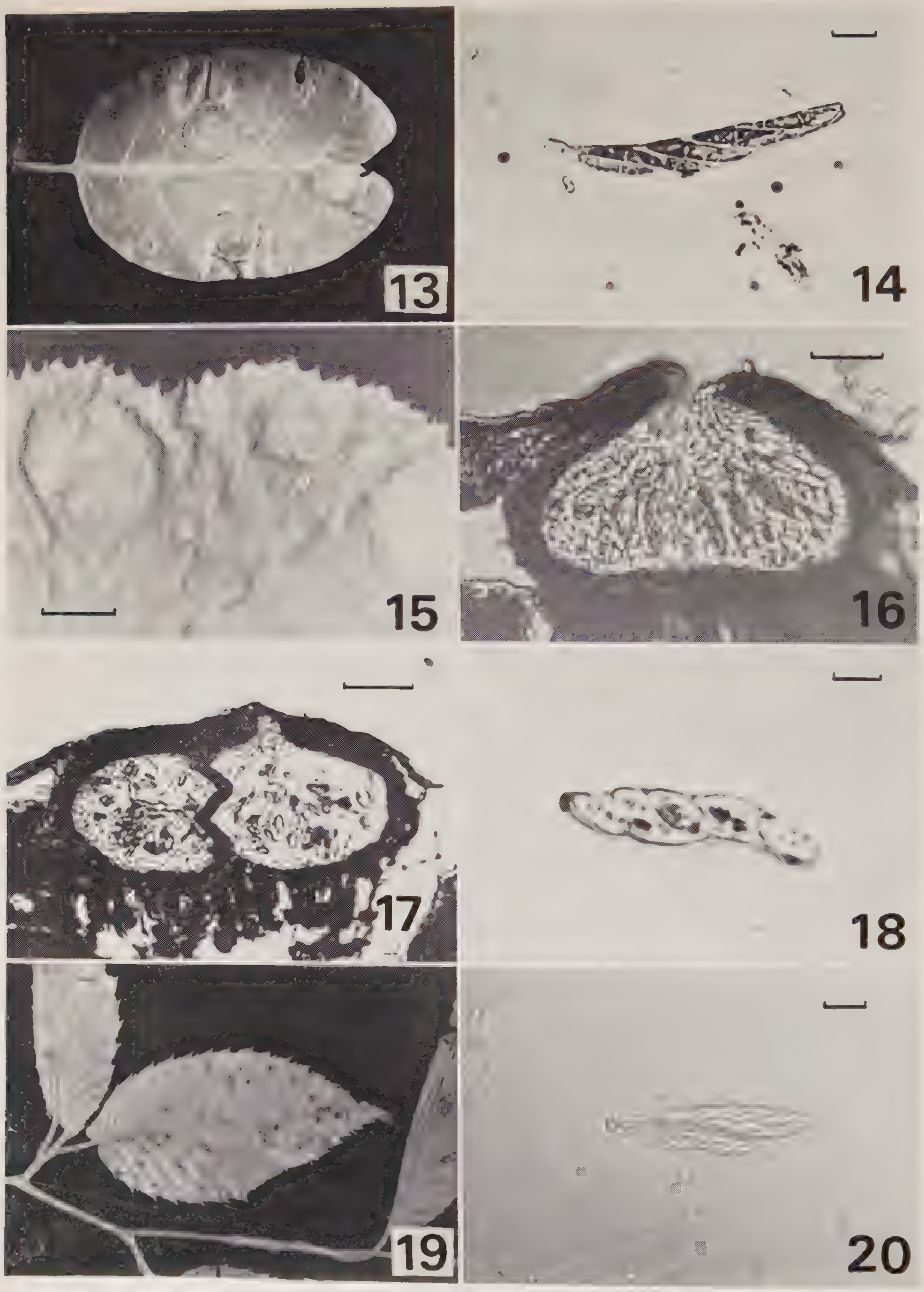


Fig. 12. *Dicarpella rubicola*: a. Perithecium. b. Ascus. c. Ascospores. (Scales: 10 μm



Figs. 13–14. *Leiosphaerella ilicis*: 13, Lesions. 14, Ascus.
Figs. 15–18. *Dicarpella rubicola*: 15, Lesions. 16, Uniloculate perithecium. 17, Conglutinated perithecia. 18, Ascus.
Figs. 19–20. *Mamiania fimbriata*: 19, Stromata. 20, Asci.
(Scales: 14, 18 and 20, 10 μm. 15, 5 mm; 16 and 17, 30 μm)

Barr by shorter ascospores. The conidial association of *D. rubicola* has not yet been observed.

13. ***Mamiania fimbriata*** (Pers. ex Fr.) Ces. et de Not., Comm. Soc. Crit. Ital. **1**: 210. 1863. (Figs. 19, 20)

≡*Sphaeria fimbriata* Pers. ex Fr., Syst. Mycol. **2**: 436. 1823.

≡*Gnomonia fimbriata* (Pers. ex Fr.) Auersw. apud Rabh., Fung. Europ. 928. 1866.

≡*Gnomoniella fimbriata* (Pers. ex Fr.) Sacc., Syll. Fung. **1**: 419. 1882.

Spots circular to irregular in shape, at first yellowish brown, later chestnut brown; stromata immersed in the leaf tissue, surrounded by the black crust, scattered or gregarious, up to 1 mm in diam, frequently coalesced and large; perithecia mostly hypophyllous, globose to depressed globose, 1 to several in a stroma, 400–500 μm in diam, with a long neck which is protruding from the leaf surface and 250–300 μm long; asci oblong, with a short stipe, obtuse and with a refractive apical annulus at the apex, 38–46 × 7.5–9 μm; ascospores biseriate, elliptic to oblong, 1-septate near the proximal end, not constricted, hyaline, 9–11 × 3–4 μm.

Specimen examined: On the living leaves of *Carpinus japonica* Bl. (*Kumashide*). Mt. Takadake, Ato-cho, Abu-gun, Yamaguchi Pref. (Oct. 10, 1974. K. Katumoto and N. Miake-YAM 22628).

Distribution: Europe, North America and Japan (Honshu).

In Japan, this species was first recorded by Hara (1923) on *Carpinus laxiflora* Bl. in central Honshu, and later by Yamamoto and Tatsuyama (1956) on *C. tschonoskii* Maxim. in Shimane Prefecture of western Honshu. Kobayashi (1970) noted in his work on the Diaporthaceae of Japan that no material of this fungus in Japan had been able to examine. Barr (1978) noted that the fungus is common although inconspicuous in living leaves of *Carpinus*. This fungus seems to be also widely distributed throughout the temperate zone of Japan. *Carpinus japonica* is a newly recorded host plant for this fungus.

The author thanks Dr. Iwao Hino, Ube Junior College, Mr. Nagato Miake, Ogori Junior High School in Yamaguchi Prefecture, and Dr. Yukio Harada, Hirosaki University, for their kindness providing with the fungal materials.

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摘　　要

西日本産植物寄生子嚢菌類として，4新種を含む核菌綱の7種を病名を付して記述する.

Phyllachora ficuum Niessl イヌビワ黒やに病. 西日本には広く分布し，ふつう葉表の角皮下に子座を形成する. 同じくイヌビワに寄生し，葉裏の表皮あるいは柔組織内に子座を形成するとされている *Catacauma nipponicum* H. et P. Syd. は本種の異名と考えられる.

Phyllachora fici-wighlianae Sawada アコウ黒やに斑点病. *P. ficuum* とは，大型で不定形の子座あるいは狭いだ円形の子嚢胞子の形態などから明らかに区別される. 九州南部と台湾に分布.

Hyponectria dicalycis Katumoto（新種）クロキ輪状黒点病（新称）. 葉裏に生ずる子嚢殻は，しばしば白色の中心部をもった輪状黒色の細点状を呈する.

Leiosphaerella dicalycis Katumoto（新種）クロキ黒点病（新称）. 葉裏にやや突出した小黒点状の子嚢殻を形成する.

Leiosphaerella ilicis Katumoto（新種）ソヨゴ褐紋病（新称）. 葉に円形，褐色のはっきりした病斑を形成し，その表面に子嚢殻を散生する.

Dicarpella rubicola Katumoto（新種）フユイチゴ褐斑病（新称）. 葉に大型でやや不整形の病斑を形成し，多数の子嚢殻を散生する.

Mamiania fimbriata (Pers. ex Fr.) Ces. et de Not. クマシデ黒斑病. 日本では今までアカシデおよびイヌシデ上に知られていて，クマシデは本種の宿主として初記録である. 日本での記録はまだ少ないが，温帯地域に広く分布しているものと思われる.

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Microfungi Japonicae
I. Nova species *Cirrenaliae* in cortice *Abietis homolepidis*

Junta SUGIYAMA*

杉山純多*：日本産微小菌類. Ｉ. ウラジロモミ樹皮上の
Cirrenalia 属の１新種

Summary

Cirrenalia japonica Sugiyama sp. nov., a helicosporous dematiaceous hyphomycete occurring on cortex of *Abies homolepis* Sieb. et Zucc. in Japan, is proposed. The semi-helicoid conidia are dictyoseptate, tightly 1/2 to 1-coiled, and the apex of monoblastic, determinate conidiogenous cell and the base of conidia are found usually at the periphery of the coil.

During a survey of the microfungi of Japan an interesting dematiaceous hyphomycete was found on cortex of *Abies homolepis* Sieb. et Zucc. at Karuizawa (alt. ca. 950 m), Kitasaku-gun, Nagano Prefecture, Japan in 1976 (August) and also in 1980 (October). Twelve collections of this fungus are assigned to a new species of *Cirrenalia*: *C. japonica* Sugiyama sp. nov., for which description and illustrations are provided here.

The holotype and paratype collections of *C. japonica* are maintained at the Mycological Herbarium, National Science Museum, Tokyo (TNS-F). The isotype and paratype collections are also deposited in the National Mycological Herbarium, Biosystematics Research Institute, Canada Department of Agriculture, Ottawa (DAOM), and in the Mould Herbarium, Institute of Applied Microbiology, University of Tokyo, Tokyo (Herb. IAM-F).

Description

Cirrenalia japonica Sugiyama, sp. nov. Figs. 1–2.

Coloniae dispersae, pulvinatae, punctiformiae, atro-rufobrunneae vel atro-brunneae, laxae, nitidae, usque ad 250 μm diam.

Mycelium immersum vel superficiale, ex hyphis ramosis, laevis, tenuitunicatis, brunneis, 2.5–5.5 μm crassis compositum.

Stroma nullum vel rudimentale.

Conidiophora fasciculata, simplicia vel ramosa, pallide brunnea, septata, laevia,

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usque ad 63 μm longa, 2.5-6 μm crassa, ex cellulis inflatis usque ad 12 μm crassis saepe composita.

Cellulae conidiogenae monoblasticae, in conidiophoris in corporatae, terminales, determinatae, saepe inflatae.

Conidia acrogena, solitaria, sicca, semi-helicoidea, arcte 1/2 ad semel contorta ad maturitatem, laevia, rufobrunnea, septis transversalibus 4–9 et plerumque septis longitudinalibus et/vel obliquis 1 vel pluribus praedita, non vel leniter constricta, ad apicem obtusa, cum cellula basali pallide brunnea et conico-truncata; conidia semicircinata, plerumque 18–28 μm diam; filum ad partem latissimam (vel ad apicem) 12-20 μm crassum.

Habitat: In cortice *Abietis homolepidis*, Japonia.

Holotypus: In cortice *Abietis homolepidis*, "Karuizawa (alt. ca. 950 m), Kitasaku-gun, Nagano Pref.," Japonia, 27.viii.1976, leg. J. Sugiyama numero 76-67(b), TNS-F-234471.

Colonies scattered, pulvinate, punctiform, dark reddish brown to blackish brown, loose, shining, up to 250 μm diam.

Mycelium immersed or superficial, composed of branched, septate, thick-walled, brown hyphae 2.5–5.5 μm wide.

Stroma none or rudimentary.

Conidiophores fasciculate, simple or branched, pale brown, septate, smooth, up to 63 μm long, 2.5–6 μm wide, often with inflated cells up to 12 μm wide.

Conidiogenous cells monoblastic, integrated, terminal, determinate, often inflated.

Conidia acrogenous, solitary, dry, tightly 1/2–1-coiled at maturity, smooth, reddish brown, with 4–9 transverse septa and mostly one or more longitudinal and/or oblique septa, not or slightly constricted at the septa, apex obtuse, basal cell pale brown and conico-truncated; semi-coiled conidia commonly 18–28 μm diam; the filament is 12–20 μm wide at the broadest part (or at the apex).

Collections examined: (1)–(5) On cortex of *Abies homolepis* Sieb. et Zucc., occasionally associated with *Nectria* sp., Karuizawa (alt. ca. 950 m), Kitasaku-gun, Nagano Pref., Japan, 27.viii.1976, leg. J. Sugiyama #76-58(a) (slides only) (TNS-F-234472, Herb. IAM-F-0001, **paratype**), #76-67(b) (TNS-F-234471, **holotype**; DAOM 176182, Herb. IAM-F-0002, **isotype**), #76-71(b) (TNS-F-234473, Herb. IAM-F slide 0003, **paratype**), #76-74(b) (TNS-F-234474, Herb. IAM-F-0004, **paratype**), and #76-75(b) (TNS-F-234475, **paratype**).

Additional collections examined: (6)–(12) On cortex of the same host at the same locality described above, occasionally associated with *Nectria* sp., 20.x.1980, leg. J. Sugiyama #80-010 (TNS-F-234476, Herb. IAM-F-0005, **paratype**), #80-021 (TNS-F-234478, Herb. IAM-F-0006, **paratype**), #80-022 (TNS-F-234477, Herb. IAM-F-0007, **paratype**), #80-024 (TNS-F-234479, Herb. IAM-F-0008, **paratype**), #80-031 (TNS-

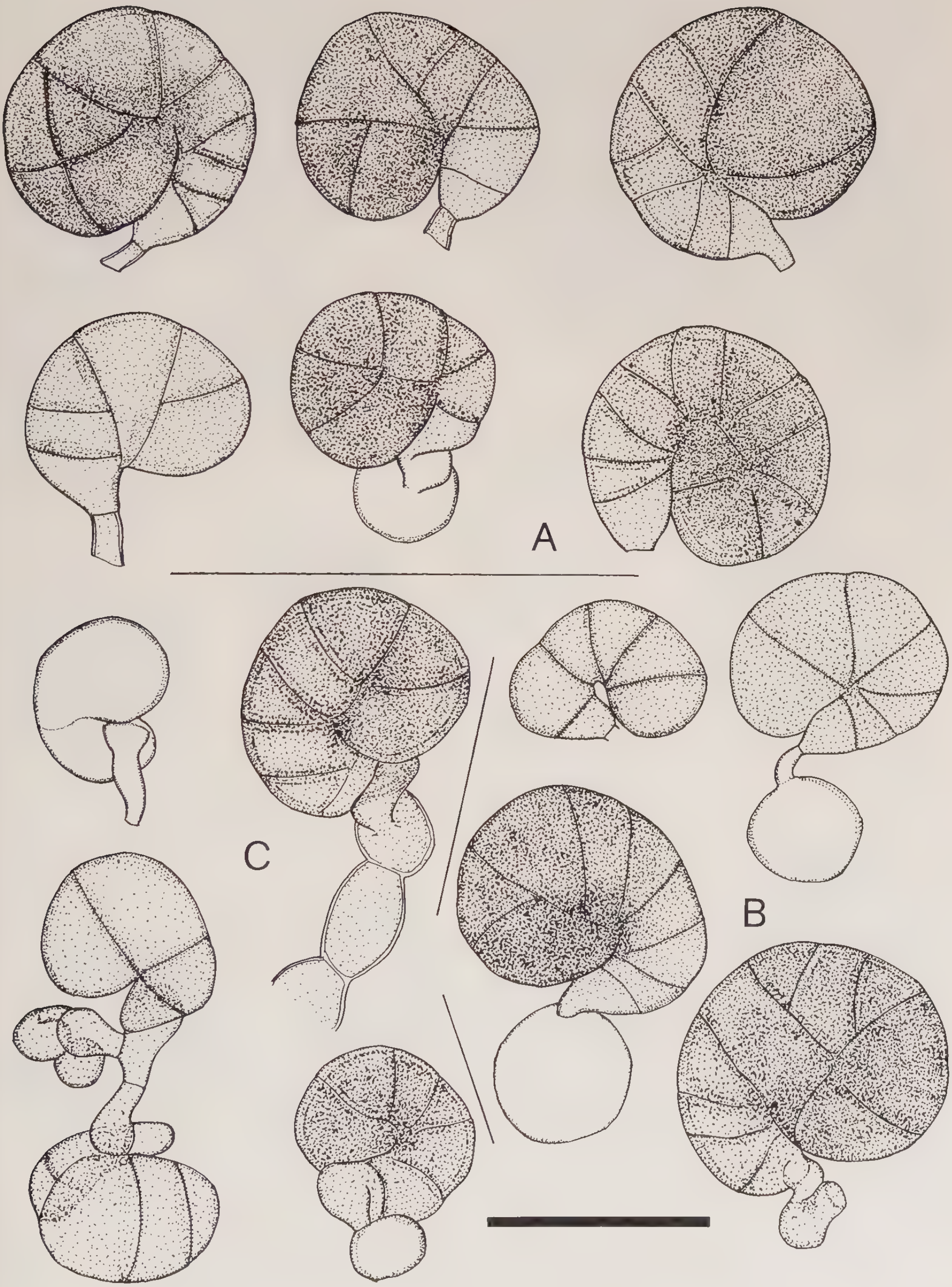


Fig. 1. *Cirrenalia japonica*; A, from TNS-F-234471 (holotype); B, from TNS-F-234472 (paratype); C, from TNS-F-234474 (paratype). Conidiophores, conidiogenous cells and conidia. (Scale: A–C, 20 μm)

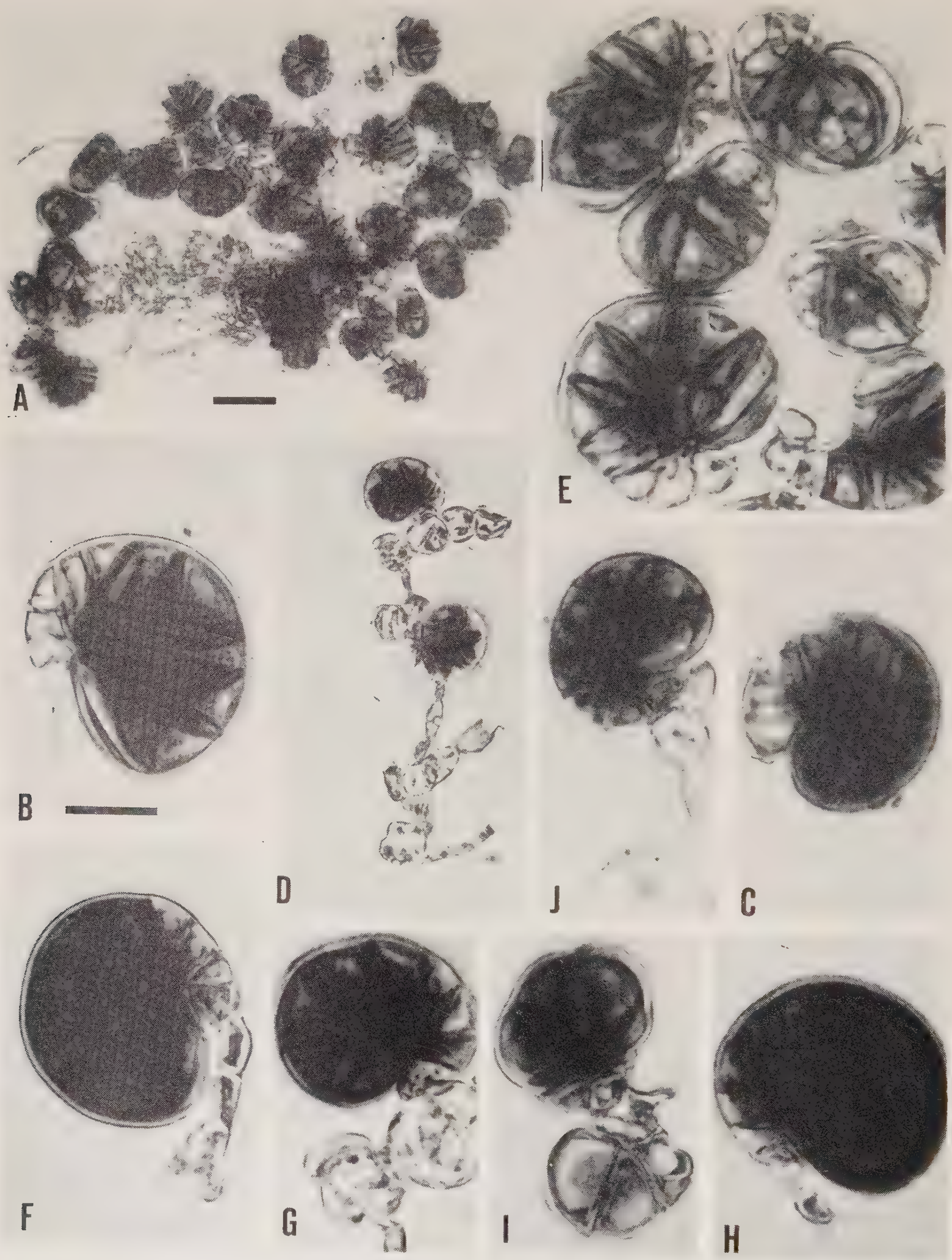


Fig. 2. *Cirrenalia japonica*; A-C, from TNS-F-234471 (holotype); D–H, from TNS-F-234472 (paratype); I–J, from TNS-F-234474 (paratype). A, Fructification. E, I, Immature conidia. B, C, F, H, Mature dictyoseptate conidia. D, G, J, Solitary, terminal, holoblastic conidia produced by determinate, inflated conidiogenous cells. (Scales: A, D, 20 μm; Others, except A and D, 10 μm).

F-234480, Herb. IAM-F-0009, **paratype**), #80-033 (TNS-F-234481, Herb. IAM-F-0010, **paratype**), and 21.x.1980, leg. J.S., #80-036 (TNS-F-234482, Herb. IAM-F-0011, **paratype**).

Parts of collections, i.e., J.S. #80-010, #80-022 and #80-036, are also preserved in Herb. DAOM (Ottawa).

In pure culture

No germination of conidia from the field material made by the author in 1976 (August) had been obtained on common laboratory media. While the conidia of additional collections gathered in 1980 (October) germinated in 3 days on malt (with yeast extract) and potato-dextrose agars at room temperature. Germination occurred generally by the emergence of a germ tube from a basal cell of the conidium. On both media, the colonies reached a diam of 1–3 mm after 1 month of incubation at room temperature but no conidia were seen. The colonies were olive grey, restricted, raised, dense, and velutinous; the reverse was black. The mycelium was superficial and immersed in the substrate, composed of branched, septate, smooth, pale brown to olive grey hyphae 2.5–4 μm wide. The stromatic aggregations of cells were formed.

Discussion

Cirrenalia, an anamorph-genus of the Hyphomycetes, was porposed by Meyers and Moore (1960) based upon the single species *Cirrenalia macrocephala* (Kohlmeyer) Meyers et Moore. In addition to the type species originally found on submerged wood of *Pinus* and *Fagus* in the Gulf of Venice, Italy, four other taxa have been assigned to the genus. These include three marine species, i.e., *C. pygmea* Kohlmeyer (1966), *C. pseudomacrocephala* Kohlmeyer (1968) and *C. tropicalis* Kohlmeyer (1968), and one terrestrial species, *C. donnae* Sutton (1973), on cortex of *Abies balsamea* in Canada.

The present generic concept of *Cirrenalia* can include this new species which produces dictyoseptate, tightly semi-helicoid conidia. Of the taxa currently assigned to *Cirrenalia*, *C. japonica* most closely resembles *C. donnae* in forming none or only a rudimentary stroma on *Abies* cortex. An examination of the holotype collection of *C. donnae* (Herb. IMI 145775) and another (IMI 145750) indicated that *C. japonica* can be distinguished from *C. donnae* by the morphology of the conidia.

C. japonica is characterized by conidia which are tightly 1/2 to 1-coiled usually with 5–9 transverse septa, and with one or more, commonly 5, longitudinal and/or oblique septa, and by the filament which is 12–20 μm thick at the broadest part. Also the apex of the conidiogenous cell and the base of conidia in *C. japonica* are found usually at the periphery of the coil.

In *C. donnae* (Fig. 3), however, the conidia are tightly 1–1.5 times coiled, transversely

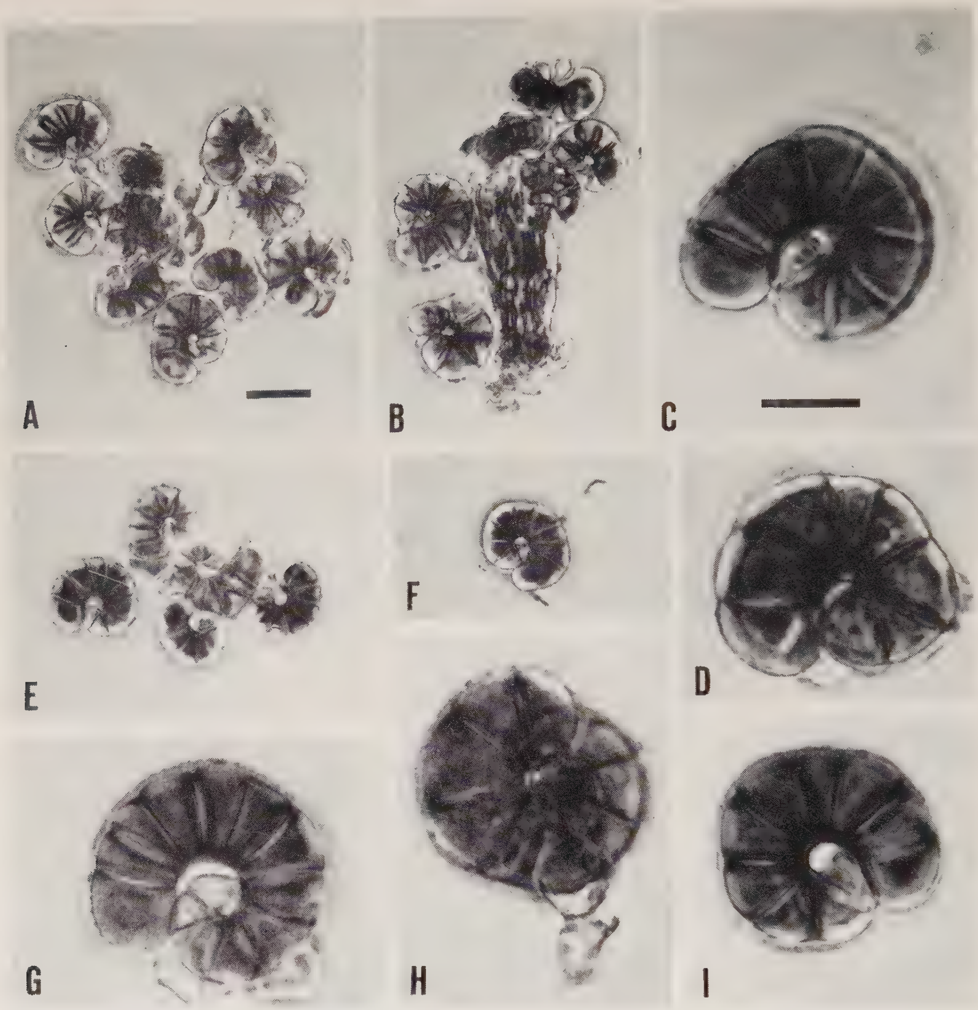


Fig. 3. *Cirrenalia donnae*; A–D, from the holotype collection Herb. IMI 145755; E–I, from Herb. IMI 145750. Helicoid conidia, with no longitudinal and/or oblique septa: The apex of the conidiogenous cell and the base of the conidium lie in the center of the coil. (Scales: A–B, E–F, 20 μm; C–D, G–I, 10 μm.)

6–12 septate, seldom with a longitudinal and/or oblique septum and the filament is 10–11 μm thick at the broadest part. Also, in *C. donnae* the apex of the conidiogenous cell and the base of conidia are usually located at the center of the coil. The differences between *C. donnae* and three other species of *Cirrenalia* have been precisely noted by Sutton (1973) and also by Ellis (1976).

The previously known distribution of *Cirrenalia* spp. in Japan appears to have been limited to *C. macrocephala*, collected on *Cryptomeria japonica* and *Abies firma* wood blocks submerged in Kagoshima Bay and Iwaya (Awaji Island), respectively (Tubaki, 1966).

The author is much obliged to Dr. S. J. Hughes, Biosystematics Research Institute, Canada Department of Agriculture, Ottawa, for reviewing the manuscript critically and for correcting the English of the text as well as many helpful comments, to Dr. B. C. Sutton, Curator of the Commonwealth Mycological Institute (Herb. IMI), Kew, for the loan of type material of *Cirrenalia donnae* and *C. tropicalis*, and to Prof. Masatomo Suzuki, Biology Laboratory, Ibaraki University, Mito, for identifying the host plant of the specimens.

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摘　　要

日本産ウラジロモミ (*Abies homolepis* Sieb. et Zucc.) の樹皮上に見出した渦巻状，暗色不完全菌を *Cirrenalia* 属の新種 *C. japonica* Sugiyama として記載した．本菌の半渦巻状分生子は多隔壁，かつ 1/2～1 回渦を固く巻き，加えて単分芽型，有限型の分生子形成細胞と分生子の基部細胞はともに通常渦巻の外周に位置する特徴が認められた．

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日菌報 (Trans. mycol. Soc. Japan) 22: 54, 1981

新 刊 紹 介

Dickinson, C., and J. Lucus: The Encyclopedia of Mushrooms. 280p. & 658 figs. Orbis Publishing Ltd., London. 1979. £ 9.95.（価格 6,700円）東京銀座イエナ書店扱（Phone 03-571-2980).

この本は1頁平均2.4図と図版が多く，楽しいキノコの本といえます．さらにA5版の1頁分，または1.5頁にわたるような，大きな図版は見る人にとってキノコへの興味を必ず起こさせます．とびらに書かれている紹介文にも，The Encyclopedia of Mushrooms opens this fascinating world to the general reader more completely than ever before……… と一般の人々への啓発書であることを明らかにしています．内容も多方面におよんでいて，前半は117頁にわたって，歴史における菌類，その生物学，生態，発生シーズン，発生地，毒キノコ，カビの利用，食物としてのキノコ，培養法，同定法などキノコを多角的にとらえて，ごく平易に書かれています．後半は図鑑としての Reference section が147頁で，この中には子のう菌類40種，担子菌類腹菌類35種，異担子菌類9種，ヒダナシタケ目64種，ハラタケ目368種，計516種がカラー写真で示され，解説が付されています．その他一般索引，文献紹介などの他に，用語解説として126語，Common English names の索引196種がのっていて便利です．

著者2人はいずれも Open University の adult education で mycology を教えていると後とびらに書かれており，このような本をあらわすのに適格な学者であると考えられます．Dr. Colin Dickinson は Newcastle-upon-Tyne 大学の教授で，英国菌学会の Programme Secretary として活躍しています．Dr. John Lucas は Nottingham 大学の plant biologist として研究活動を行っています．

特に興味を引かれる内容は Coprophilous Fungi や Hallucinogenic Mushrooms のまとめられた記述です．前者ではミズタマカビの胞子のう柄が光の方を向き黒い胞子のうをジェット噴射のようにはじき飛ばす模式図はアイデアに富んでいますし，内容も多くの菌類を取りあげておもしろく，後者では中央アメリカの風習が興味深く伝えられています．また A Collector's Code という，食用キノコ集めのための忠告が7項目にわたって書かれています．このうちつぎの2項目は日本の手引書にないものですが，採集にあたって留意すべきことでしょう．3. Always pick all of the mushroom. 4. Avoid any old, damaged or decaying specimen. 残念なことに一般向きのため，胞子，シスチジアなどの顕微鏡図が少なく，科ごとのこまかい検索表もないので，十分な同定の目的には不便なことです．

以上の点はありますが，邦書には類例のない菌類全般に関する解説書，図鑑で，まさに百科辞典です．アマチュア，研究者を問わず座右に備えて，参考にしたり，楽しんだりする価値のある本として推賞します．

(Koichi Nunomura　布村 公一)

Trans. mycol. Soc. Japan (日菌報) **22**: 55–59, 1981

An undescribed species of *Bactridium* and its cultural studies

Keisuke TUBAKI* and Toru OKUDA**

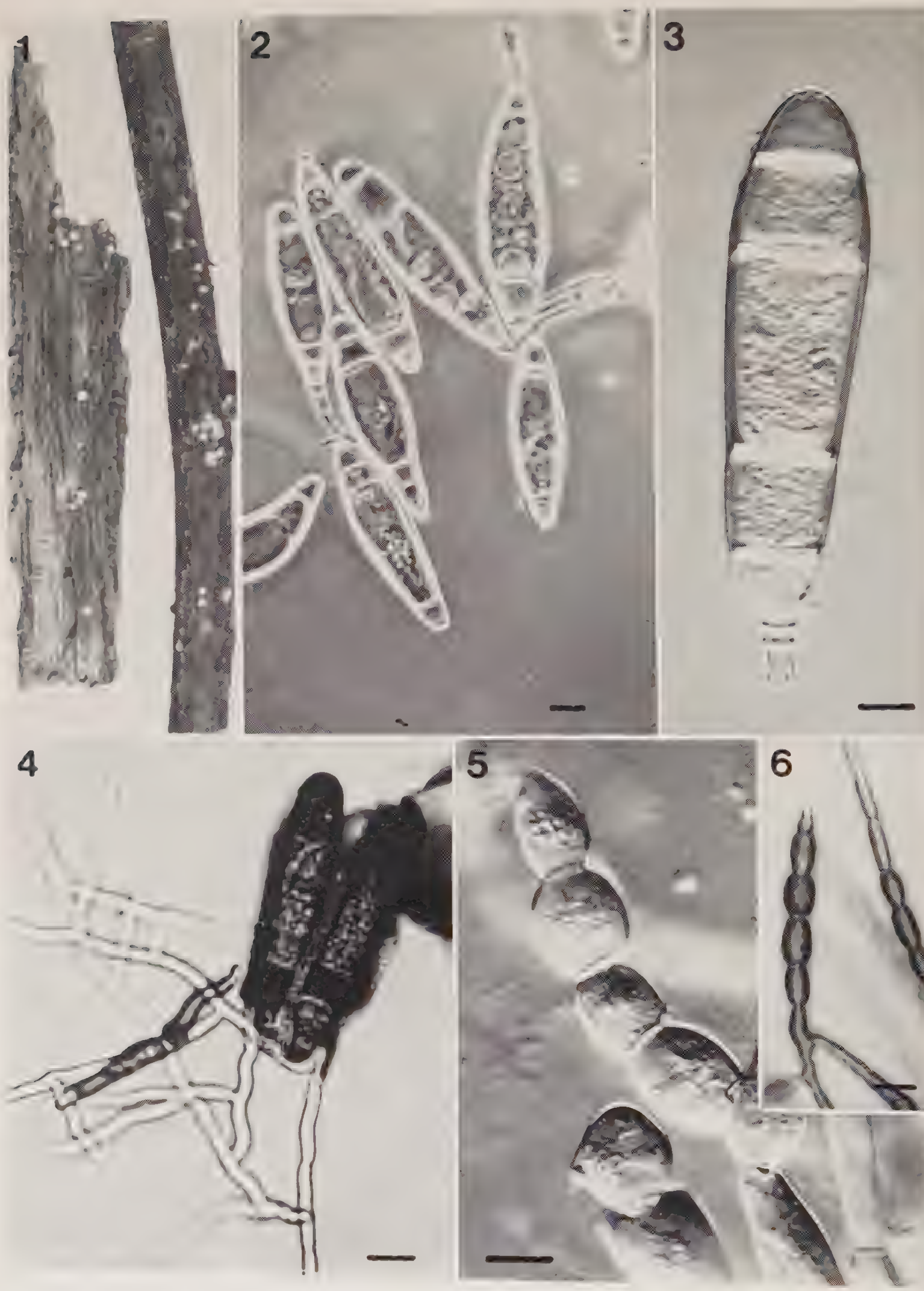
椿 啓介*・奥田 徹**：*Bactridium* の1新種とその培養世代

Summary

A new species of the hyphomycete genus *Bactridium*, *B. subglandis* Tubaki, is described and characteristics of its culture are given.

Since the paper presented by this author (K. Tubaki) on the Japanese species of *Bactridium* in 1970, there have been no subsequent reports on this genus. Neither have there been any cultural studies since germination of the conidia occurs extremely rarely. In the previous paper, *B. clavatum* Berk. & Br., *B. fulvellum* Berk. and *B. flavum* Kunze ex Fr. were described. In addition, the paper discussed *Bactridium* sp. 11277 collected at Mt. Tsurugi, Kagawa Prefecture which is clearly differentiated from other four-septate spored species by its larger conidia. Because of this single collection, a full description of this apparently new species was delayed until additional collections had been made. In 1977, a small collection of a *Bactridium* fungus was obtained from the decayed wood of a *Quercus crispula* near Tsuta, Aomori Pref. The conidia were four-septated and identified to the Tsurugi specimens. Subsequently, a larger number of collections were made from decaying twigs of a *Q. crispula* growing in the forest of the Sugadaira Montane Center, a branch of the University of Tsukuba, in Sugadaira, Nagano Pref. (Fig. 1). Bright yellow sporodochia were found over the entire surface of the twig. Although germination of the conidia of this *Bactridium* species is generally very rare, a few conidia of the Aomori collection were observed to germinate by one of the authors, and the fungus was immediately isolated. Germ tubes grew out from the distal ends of the conidia (Fig. 4) and soon branched, septated, and 9–10 μm in diam, and hyaline. The mycelial growth is very reduced, flat and spreads with no visible aerial hyphae. Because there have as yet been no reports concerning the culturing of *Bactridium*, the following experiments were performed to determine the cultural charcteristics, the effect of different

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Figs. 1–6. *Bactridium subglandis* TKBF-1001.

1. Habit. 2–3. Conidia. 4. Germination of conidia. 5–6. Torulose cells. (Scales: 2, 4, and 6=40 μm; 3 and 5=20 μm)

media on growth, the utilization of carbon sources, the utilization of nitrogen sources and possible vitamin deficiencies. The purpose of this paper is to describe this new species of *Bactridium* and to report on its cultural characteristics.

Bactridium subglandis Tubaki, sp. nov. (Figs. 1–6)

Sporodochia dispersa vel aggregata, hemisphaerica, ad 1 mm diam, pallide lutea vel aurantio-brunnea. Mycelium immersum, ex hyphis, ramosis, septatis, hyalinis compositum. Conidiophora densa, ramosa, septata, hyalina, ad 200 μm long., ad basim 8–10 μm cr., dein 5–8 μm. Conidia ellipsoidea, crasso-tunicata (ad 3–4 μm), plerumque 4-septata, cellulis binis centralibus longioribus, (180–)210–270(–290) × 42–60(–66) μm.

Sporodochia scattered or gregarious, hemispherical, up to 1 mm in diam, 0.3–0.5 mm in height, pale yellow to orange brown. Mycelium immersed, consisting of branched, septate, hyaline hyphae. Conidiophores crowded, developing stromatic compact cushions of basal hyphae, up to 200 μm long, 8–10 μm wide at the bases, tapering gradually to 5–8 μm in width and expanding to 10–12 μm in width just below the conidial attachment. Conidia thallic, forming as the blown-out end at the apex of conidiophores, ellipsoidal or clavate, rounded at the distal end, truncate at the base, predominantly four-septate; central cells largest, (180–)210–270(–290) × 42–60(–66) μm. with or without a separating cell, rarely constricted at septa, hyaline to pale orange-yellow; separating cells cylindrical when present, 6–10(–15) μm long.

Hab.: Decayed wood of *Quercus crispula*, Mt. Tsurugi, Kagawa Pref., Aug. 24, 1965 (Ex. Herb. Institute for Fermentation, Osaka, H-11276 & 11277); decayed wood of *Q. crispula*, Tsuta, Aomori Pref., Oct. 4, 1977; decayed twigs of *Q. crispula*. Sugadaira, Nagano Pref., July 22, 1980 (Ex. Herb. TKBF-1001, HOLOTYPE).

Dried type specimens are preserved in the herbarium of the Institute of Biological Sciences of this university. Cultures derived from the materials collected in Aomori have been preserved in the mycological laboratory of above university.

The present species can be differentiated from other four-septate species of the genus by its larger conidia.

Although the authors have succeeded in culturing a species of *Bactridium* axenically on a yeast-decoction agar medium, common laboratory culture media have been known to fail to support growth. Therefore, experimental studies of the nutritional conditions necessary for the fungus to grow were carried out.

Cultural characteristics

Effects of different media on growth Only mere traces of growth of *B. subglandis* occurred using common laboratory media such as malt-extract agar or potato-dextrose agar which usually adequate for most non-parasitic fungus cultures. Growth

on malt-extract agar was very reduced, with only a trace of mycelial development. On potato-dextrose agar, the same results were obtained, and on corn-meal agar, growth was extremely repressed. The best mycelial development was obtained on yeast-decoction agar (commercial brewer's dried yeast–"ebios" 5 g, glucose 20 g, KH_2PO_4 1 g, agar 15 g, distilled water 1,000 ml). On this medium, growth was still slow but spreading, and pale yellow in color. It consisted of branched and septate mycelium accompanied by a torulose cell produced at each end (Figs. 5–6). The torulose cells which were composed of several globose cells, developed blastically. Each cell was 24–33 μm in diam. The reverse of the colony is yellowish brown. No thallic conidia developed at all during the culturing process.

Utilization of carbon sources The possible utilization of sugars and of carbon sources were tested using casamino acids as possible nitrogen sources. The 19 carbon sources used were: glucose, galactose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, D-xylose, L-sorbose, rhamnose, ethanol, glycerol, erythritol, mannitol and inositol. Each carbon source was used at a rate that supplied 10 g of carbon per liter of basal medium*. The fungus was grown in a constant temperature of 25 C for 25 days. As a result, glucose, D-xylose and ethanol were utilized slowly. Sucrose, maltose and cellobiose were utilized but only weakly, and the other carbon sources did not support growth at all but did not inhibit the production of mycelium, which were, however, very reduced.

Utilization of nitrogen sources The possible utilization of sources of nitrogen was tested, using 1% of glucose as the carbon source. The 18 nitrogen sources used were: $(NH_4)_2SO_4$, KNO_3, glycine, alanine, serine, aspartic acid, cysteine, methionine, proline, glutamic acid, histidine, valine, leucine, phenylalanine, tyrosine, isoleucine and arginine. Each of them was added at a concentration of nitrogen equivalent to 0.04%. After 25 days at 25 C, the only cultures showing significantly greater growth than the controls were those receiving the most of amino acids, except in the case of cysteine which, in fact, inhibited growth. When inorganic compounds were replaced with a single amino acid, growth was very reduced. Considerable amounts of a melanin-like compound were produced when tyrosine was used. From this, it was deduced that one or more of these amino acids may serve as the sole source of nitrogen.

Vitamin deficiency The following vitamins were tested singly and in certain combinations in Petri dish agar cultures: thiamine-HCl, biotin, pyridoxine-HCl, riboflavin, Ca-panthothenic acid, folic acid, niacin, inositol and p-aminobenzoic acid. Glucose and casamino acids were used respectively as the sources of carbon and nitrogen. All results of the experiments supported the hypothesis that there are no visible differences

* Casamino acids (vitamin free) 0.4%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7aq$ 0.05%, CaCl 0.01%, NaCl 0.01%, agar 2%.

in growth among the cultures, and therefore, that there was no vitamin deficiency.

Regardless of the kind of medium, growth is always poor and mycelial, and there is no trace of conidial development. A study of the factors inducing sporulation is a subject for future research.

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Tubaki, K. 1970. Notes on the Japanese Hyphomycetes IV. Japanese species of *Bactridium*. Trans. mycol. Soc. Japan **11**: 49–52.

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日菌報 (Trans. mycol. Soc. Japan) **22**: 60, 1981

新 刊 紹 介

Deacon, J. W.: Introduction to Modern Mycology. 197 p. Blackwell Scientific Publ., Ltd., Oxford. 1980. 価格 約4,680円.

最近，海外では菌学の入門書が次々と出版され，生物学の一環として大学の課程で菌類についての十分な知識を修得されうる状況になっているのはうらやましいことである．本書も学部学生を対象とした The Basic Microbiology Series の第7巻として刊行されたものである．もちろん，シリーズの他の巻とのかね合いはあると思われるが，本書のみを購入しても菌類全般についての広い知識を身につけられるように構成されている．すなわち，その内容は次の14章からなっている．

1. Introduction. 2. Structure and fine structure. 3. Growth. 4. Differentiation. 5. Nutrition. 6. Metabolism. 7. Environmental conditions for growth: tolerance of extremes. 8. Genetic systems. 9. Spores and their dispersal. 10. Fungi as saprophytes. 11. Fungi as saprophytes: microbial interactions. 12. Fungi as plant parasites. 13. Fungi as animal parasites. 14. Prevention and control of fungal growth.

これらのタイトルからも想像されるように，著者は前半で生理を中心に菌類が他の生物とどのように違うのか，いわば菌類自身の特質を解説し，後半では菌類と他の生物とのつながり，菌類が自然界で示す生物活性を中心に，実際的な面を述べている．通り一遍になり勝ちな記事を豊富な図，写真や表を挿入し，学生の理解を深めるように工夫されている． Modern という名にふさわしく，菌類についての最近の進歩をよく follow up しているが，これだけ多岐にわたる分野を compact にまとめるとなると個々の内容がどうしても簡単にならざるを得ないのは仕方無い．そのことを意識して，巻末に各章ごとの参考図書を列挙してあり読者の指針になるよう配慮されている．

欧米の教科書，一般参考書が日本に比べてすぐれている点の1つはバランスのとれた内容にある．日本の科学教育がとかく応用分野に重点を置く傾向があるのに対し，欧米では広く基礎知識を身につけて卒業し，深い専門分野は学部（大学）を卒業してからという考えの違いがある．カビの示す典型的な特性ともいえる二次代謝については，本書ではきわめて簡単に説明され，主要例としてペニシリンとアフラトキシンが挙げられているのみであるが，反面糞生菌類や淡水不完全菌類を例にとった spore dispersal の記述が図解とともに示されている．こうした応用に直結しない題材については，一部の研究者の興味に留まっているのが日本の実情である．教養としての菌学において，これだけの内容が授けられるならば，いうことないといった印象をうけた．著者の Edinburgh 大学だけでなく，本書が世界中の各大学において教科書として受け入れられるであろうことは予想に難くないところであり，菌類の認識を広める上できわめて有意義な出版といえよう．

専門が非常に細分化された現代では，自分の専攻分野以外のことを再勉強する上で，こうした適切に要約された基礎知識を与えてくれる書籍が社会人にとっても必要であり，この機会にちらっとでも覗かれてみてはいかがでしょうか．

(Shun-ichi Udagawa 宇田川俊一)

Trans. mycol. Soc. Japan (日菌報) 22: 61–63, 1981

A new species of *Exobasidium* parasitic on *Symplocos theophrastaefolia*

Shôichi HIRATA*

平　田　正　一*：カンザブロウノキに寄生する餅病菌の1新種

Summary

At the conclusion of the comparisons especially with *Exobasidium symploci* and *E. symploci-japonicae*, the *Exobasidium*, which is growing on *Symplocos theophrastaefolia* in Miyazaki Prefecture, is described as a new species, *E. sakataniense* S. Hirata.

On September 8, 1949, in an evergreen broad-leaved forest situated in a moist valley at Sakatani, Nichinan-shi, Miyazaki Prefecture, Japan, the writer first collected leaf spots of *Symplocos theophrastaefolia* Sieb. et Zucc. (Japanese name: Kanzaburô-no-ki), which were caused by a species of the genus *Exobasidium*. After that materials of the same fungus were sometimes obtained by the writer at other localities in Miyazaki Prefecture.

Diagnosis of the leaf spots is as follows. Spots occurring in early summer to late autumn, developing on opening buds, young leaves and stems, causing the leaf curl and distortion when formed on the whole leaf surface, at first pale yellow and small, later becoming circular and subcircular to irregular, yellowish brown with a deep violet halo, blister-like, 1–7 mm in diam, concaved on the upper leaf surface and protruding on the lower one, swelling about 2 times thicker than the healthy part of host plant by the cell hypertrophy without the cell division. Hymenium hypophyllous, white, forming a continuous layer on the spot. Basidia emergent through the intercellular portion of epidermis, roughly projecting about 33–55 μm beyond the cuticle, and 5–8 μm wide, hyaline. Sterigmata ordinarily 2 per basidium, hyaline, 5–9 μm long, 3–4 μm wide at the base. Basidiospores cylindrical, slightly curved, narrowed toward the base, hyaline, 3-septate at maturity, rarely 2-septate, germinating with germ-tube, $19–28 \times 4–6$ μm (average 24.4×5.1 μm). Conidia cylindrical, rarely tapering toward the base, hyaline, $7–18 \times 1.5–2$ μm.

Culture note: Spores germinated easily on PSA and the colony was yeast-like, white, turned dull gray with age, and crinkled in its surface with a few short aerial hyphae.

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According to the literatures reported in the past, two species, viz. *Exobasidium symploci* Ell. et Mart. (Saccardo, 1891) and *E. symploci-japonicae* Kusano et Tokubuchi (Kusano, 1907), were considered as the closely related species to the present fungus. However, *E. symploci*, parasitic on *Symplocos tinctoria*, forms the distortion of buds and flowers of the host plant and whose basidiospores (15–21 $\times$ 2 μm) are distinctly smaller than those of the present fungus. *E. symploci-japonicae*, which is growing on *Symplocos lucida* Sieb. et Zucc., causes remarkable gall-like hypertrophy (Ito, 1955), and having 4 sterigmata per basidium and longer basidia (120–140 μm long) and smaller basidiospores (17–22.5 $\times$ 6–7 μm) compared with those of the present fungus. Therefore, the writer is inclined to regard the present fungus as a new species.

The scientific name of the fungus, *Exobasidium sakataniense*, was chosen in commemoration of its first collected locality, Sakatani in Miyazaki Prefecture.

Exobasidium sakataniense S. Hirata, sp. nov. (Figs. 1–2)

Maculis in foliis numerosis rotundis vel subrotundis, plerumque 1–7 mm diam, margine purpureifusco, suprasuperficie flava vel pallide-brunnea, subtus leviter protrudentibus; hymeniis hypophyllis, albofarinosis; basidiis evolventibus in hymeniis, clavatis vel cylindricis, hyalinis, continuis, 30–55 $\times$ 5–8 μm, terminaliter cum 2 sterigmatibus, quibus longiconicis 5–9 $\times$ 3–4 μm; basidiosporis falcatis vel longifusiformibus, leviter curvatis ad basim, hyalinis, continuis, 19–28 $\times$ 4–6 μm, maturitate 2–3-septatis; conidiis lateralibus vel terminalibus, anguste cylindricis, hyalinis, 7–18 $\times$ 1.5–2 μm.

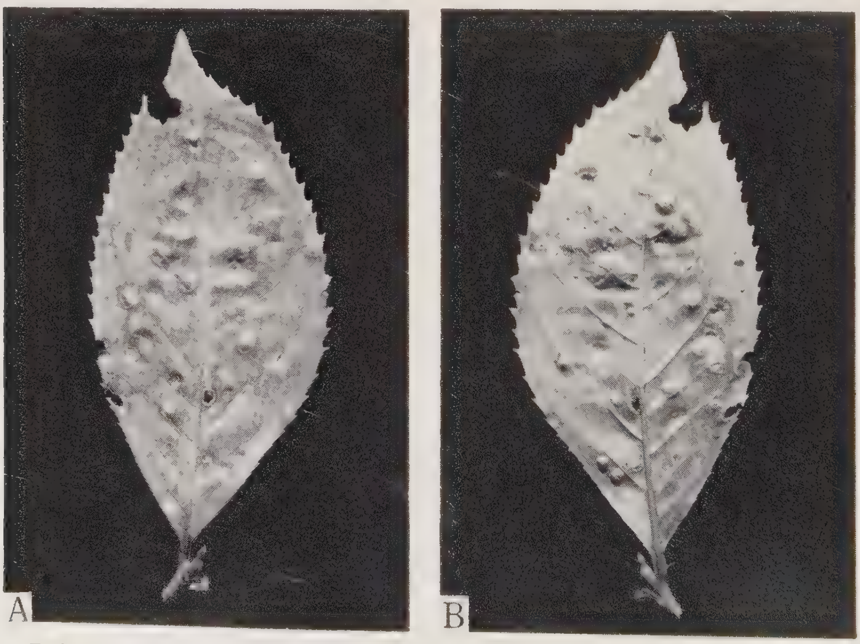


Fig. 1. *Exobasidium sakataniense*: upper (A) and lower (B) surfaces of infected leaves showing the spots.

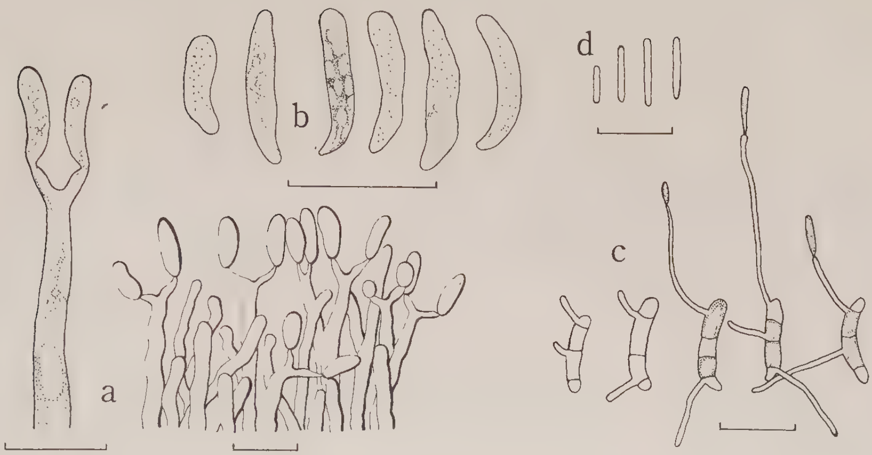


Fig. 2. *Exobasidium sakataniense*: a. basidia and basidiospores, b. basidiospores, c. germination of basidiospores, d. conidia (each scale: 20 μm).

Specimens examined: On leaves and stems of *Symplocos theophrastaefolia* Sieb. et Zucc. (Kanzaburô-no-ki)—Mt. Boroishi, Miyazaki-shi, Miyazaki Pref., Oct. 22, 1980, by H. Otsubo (**Holotype**, MAPL-2); Sakatani, Nichinan-shi, Miyazaki Pref., Sept. 8, 1949, by S. Hirata; Aoidake, Tano-chô, Miyazaki Pref., Oct. 13, 1963, by S. Hirata; Hôrigawa, Kitagawa-chô, Miyazaki Pref., July 13, 1951, by S. Hirata; Mt. Boroishi, Miyazaki-shi, Miyazaki Pref., Aug. 29, 1979, by S. Hirata.

Distribution: Japan (Miyazaki Prefecture).

Japanese name: Kanzaburô-no-ki no mochibyô-kin (カンザブロウノキの餅病菌).

The type specimen is deposited in the Mycological Herbarium of the Faculty of Agriculture, Miyazaki University.

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Saccardo, P. A. 1891. Sylloge Fungorum **9**: 244.

摘　　要

宮崎県日南市酒谷において常緑樹のカンザブロウノキの若芽と成葉に寄生する *Exobasidium* 菌の1種を採集し，その後，同県内で時々同菌を得た．葉の病斑は初め淡黄色，円形，後に黄褐色，径 1–7 mm となり，破れて抜ける．新芽では，激しい変形とい縮を起して枯死する．本菌は担子体上に常に2胞子を形成するのに対して，ハイノキ科に寄生する *Symplocos tinctoria* 上の *Exobasidium symploci* およびクロキ上の *E. symploci-japonicae* などの既知種は共に4胞子を形成することで異なり，病徴においても既知の両種は新芽と花芽を侵し，著しい肥厚化を起すものである．これらの比較から，カンザブロウノキ上の本菌を新種と認め，*Exobasidium sakataniense* とした．

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日菌報 (Trans. mycol. Soc. Japan) **22**: 64, 1981

新　刊　紹　介

Holliday, Paul: Fungus Diseases of Tropical Crops. 605 p. Cambridge University Press, Cambridge. 1980. 価格 £55. (約 35,670円)

本書の著者はカカオの天狗巣病など熱帯作物病害の発生生態・防除の研究を行なっており，熱帯地域での作物病理に深い知識と豊富な経験をもっている．現在は Commonwealth Mycological Institute に所属している．

本書は熱帯作物の病原糸状菌の解説書であり，225属，約1,000種を収録している．各属，種はアルファベット順に配列され，それぞれについて分類形質（主に形態的特徴）を比較的詳細に記載し，必要に応じて分類学上の取り扱いの変遷についても簡潔に解説している．次に，各病原糸状菌の各宿主植物上での加害特性・病徴，疾病の発生状態および防除について記述している．重要病原菌や多犯性の菌（race や f. sp. の分化しているものを含む）については，主要作物ごとに解説されている．

本書は病原菌の形態を始めとする生物学的特徴および被害作物の病徴の要点を知る上で非常によい文献であるが，key がないため病原菌の同定，疾病の診断に利用しにくい点に難がある．記述・解説の重要な部分には，適切かつ豊富な文献が示されており，読者が容易に original を調べられるように配慮されている．巻末には，宿主作物・その病原菌と主要文献の一覧表があり，疾病の一般名（作物と病原菌の対応を含む）および宿主作物の一般名と学名のリストが付けられている．また，病原菌の学名，synonym と分生子世代の学名の index がある．

本書で言及されている病原糸状菌と宿主作物（約300種類）は熱帯特有のものに限られておらず，それぞれ温帯に分布・栽培の中心をもつものもかなりあるため，われわれ temperate mycologists にも参考になる．また，適切な図が付いていれば菌学の text ともなり得る．熱帯に分布の中心をもつ病原糸状菌は，地理的・気候的条件や，植生上での違いに基づき，生理的・形態的性質やそれらに起因する生態的特性が，温帯のものとは様相をかなり異にしている．われわれ temperate mycologists にとって，このような相違を理解することは，植物病原糸状菌の分類や生態を総括的に学ぶために大変必要なことではなかろうか．

(Yoshitaka Ono　小野　義隆)

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Saprophytic fungi isolated from woods in mangrove swamps and their wood-decaying capability

J. N. Rai, K. L. Garg and A. K. Jaitly*

Summary

Eighty-seven saprophytic fungi were isolated from wood samples taken from mangrove swamps, in Sunderban, West Bengal, India. Of the two media used, malt extract agar supports better fungal growth than Czapek-Dox agar. Salt tolerant forms were also isolated. Isolated fungi were comparatively fewer in number on the media with higher salt concentrations (3 and 6% NaCl). *Hemisartorya* spp. were isolated for the first time from wood from mangrove swamps. Fungi were also tested for their wood-decaying potential, and were classified into 3 groups:—strong, moderate and weak—based on the resulting decomposition. The rate of decomposition increases until about the forty-fifth day and decreases thereafter. Among the fungi tested, *Chaetomium globosum* caused the highest rate of wood decay.

Vast quantities of plant remains and forest litter (organic matter) which are mechanically incorporated into the body of soils, are used by many microorganisms as their sole carbon and energy source during the decomposition process. The value of the mangrove is based largely on the production of organic matter which has resulted from the fall of plant matter which then enters the estuarine system, where it forms the basis of a complex food web. The presence of extreme ecological conditions in mangrove swamps, for example, high osmotic concentrations, moisture levels and organic matter, poor aeration and its evergreen-dense state also afford an interesting habitat for the study of wood-decomposing fungi.

Wood-inhabiting fungi and their ability to decompose wood have been reported on by several workers (Savory, 1954; Kohlmeyer, 1969; Bette and Goos, 1978). The degradation by thermophiles has also been reported by Ofosu-Asiedu and Smith (1973) and Rosenberg (1978). The present study was made in order to isolate saprophytic fungi from wood specimens from mangrove swamps, in Sunderban, West Bengal, India and to estimate their average wood-decaying capability.

Materials and Methods

Fifty wood samples from the following localities in Sunderban, West Bengal, India, i.e., Bakhali, Gangasagar, Gosaba, Kakdwip, Pakhirala, Peerkhali, Sajanakhali and

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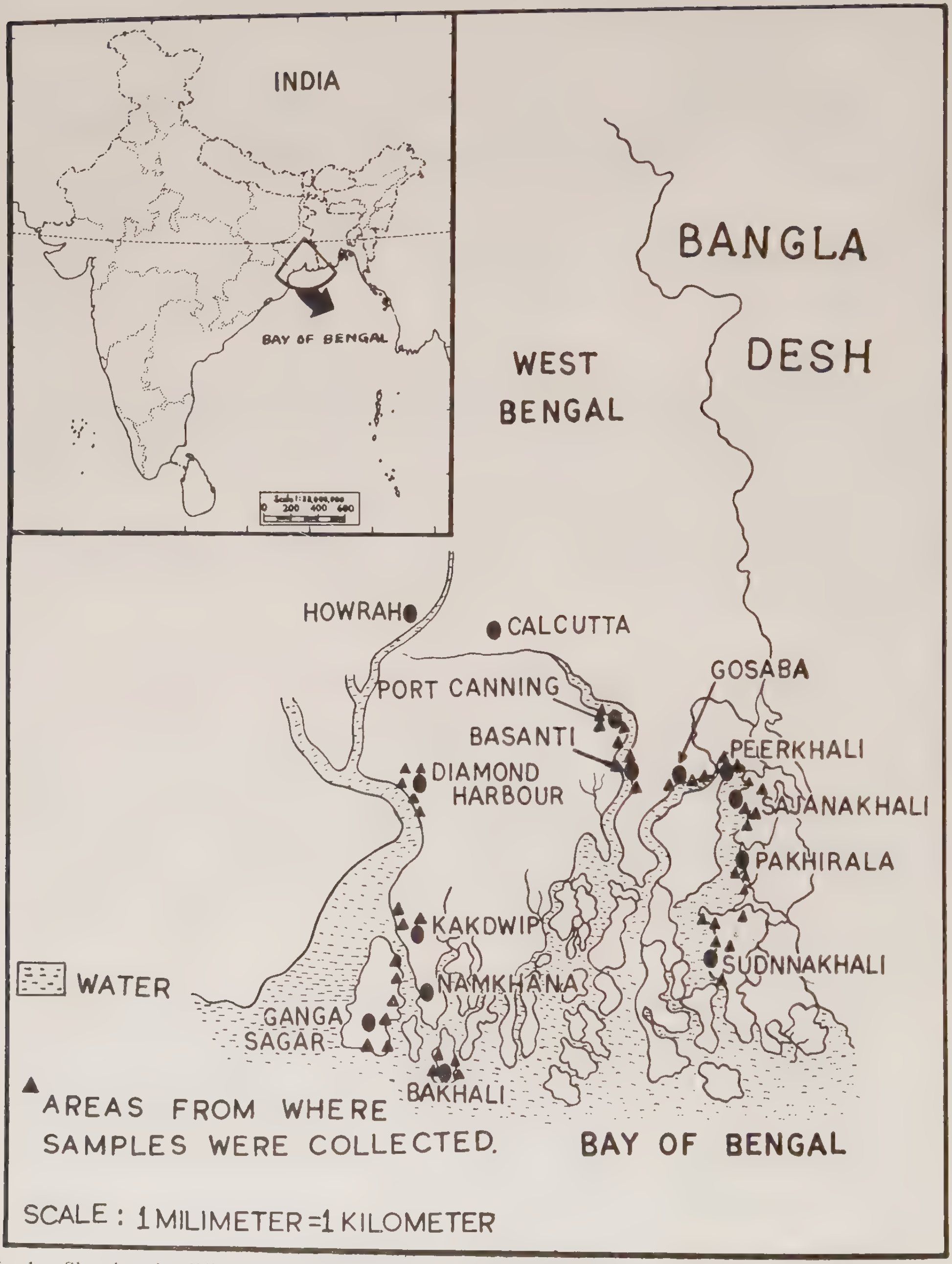


Fig. 1. Showing the different localities of Sunderban, West Bengal, India from where wood samples were collected.

Sudnnakhali (Fig. 1), were collected between Dec. 2nd, 1978 to Dec. 12th, 1978 and from Nov. 27th, 1979 to Dec. 6th 1979, and were aseptically brought to the laboratory for microbial analysis. Wood samples thus collected were divided into three categories,

viz., less decomposed, moderately decomposed, and greatly i.e., almost totally decomposed, depending upon their texture and visual appearance. The modified dilution plate and direct plate methods of Warcup (1950) were adopted for the study. Czapek-Dox agar (pH 7.0) and malt extract agar (pH 6.5) were used as media for isolation. In order to determine salt tolerant forms, the above media, supplemented with 3% or 6% NaCl, were also used. Six replicates were taken for each. Plates were incubated at 29 ± 1 C and were observed regularly after 12 h. The appearing fungal colonies were then transferred onto agar slants for taxonomic and morphological studies.

In order to examine the wood-decaying capability of fungi, decay tests were made by weight loss method. Wood blocks ($25 \times 12 \times 8$ mm) of *Tamarindus indica* were dried at 80 C to a constant weight and were sterilized by autoclaving at 15 lbs. for 30 min. The wood blocks were then transferred into sterilized 100 ml Erlenmeyer flasks containing 25 ml sterilized Czapek-Dox medium without a carbon source. Two mm discs of freshly grown mycelium were then placed in each flask. Six replicates were examined for each case.

Flasks were incubated at 29 ± 1 C for 15, 30, 45 and 60 days. After incubation, the mycelium was completely brushed off from the surface of the blocks which were dried to a constant weight at 80 C. The differences in weight as taken and percentage weight losses were calculated. Transverse and longitudinal sections were cut and examined microscopically after staining with lactophenol blue.

Results

Fungi grew more rapidly and fungal colonies were greater in number on the malt extract agar as opposed to the Czapek-Dox agar. As a result, 87 fungi representing various groups were isolated (Table 1). Of these, 4 species belonged to the Zygomycetes (order Mucorales), 18 species to the Ascomycetes (including ascosporic Aspergilli and Penicillia), 6 strains to the Mycelia Sterilia and the remainder to the Fungi Imperfecti. Of all the fungi isolated, the greatest number represented Fungi Imperfecti, indicating that it is the major group inhabiting the mangrove habitat. On media containing 3% or 6% NaCl, comparatively fewer fungi were isolated. Only *Emericella nidulans* var. *lata*, *Thielavia* spp. and *Hemisartorya* spp. representing the Ascomycetes, were isolated on the 3% media. On the 6% media, only ascosporic Aspergilli, *Emericella nidulans* var. *lata* and *Hemisartorya* spp. were found. *Hemisartorya* spp. were not isolated on normal media but occurred more frequently on the 6% media than on those with only 3% NaCl.

The Aspergilli are the dominant forms of the Fungi Imperfecti and the amounts of *Aspergillus niveus* increased as the salt concentration in the medium was raised. The species of the genera *Trichoderma*, *Pestalotiopsis*, *Curvularia*, *Fusarium* and *Penicillium* were most frequently isolated. *Cladosporium* spp. is second to *Alternaria* spp. in terms of the frequency of its occurrence, whereas *Acremonium*, *Drechslera* and *Scopulariopsis* species are

Table 1. Frequency of occurrence of saprophytic fungi from woods in Mangrove swamps (Sunderban) in malt extract and Czapek-Dox agars of different NaCl concentrations

Name	Media, Conc. of NaCl (in percentages)	Malt extract agar			Czapek-Dox agar		
		0	3	6	0	3	6
Zygomycetes							
Mucor racemosus Fres.		+++			++		
M. silvaticus Hagem		+++			++		
Mucor sp.		++			+		
Rhizopus sp.		+++			++		
Ascomycetes							
Achaetomium luteum Rai & Tewari		++					
Chaetomium arcuatum Rai & Tewari		+++			++		
C. funicola Cooke		+++			+		
C. globosum Kunze ex Fr.		++++			++		
C. indicum Corda		+++			++		
Emericella nidulans Vuill. var. *dentata* (Sandhu & Sandhu) Subram.		+++			+		
E. nidulans Vuill. var. *lata* (Fennell & Raper) Subram.		+++	++	+	++	+	
Fennellia flavipes Wiley & Simmons		++			+		
Hemisartorya maritima Rai & Chowdhery			++	++++		+	++
Hemisartorya sp.			++	++			++
Microascus manginii (Loub.) Curzi		++			+		
Neosartorya fischeri (Wehmer) Malloch & Cain		++			+		
Phoma sp.		++			+		
Sporotrichum sp.		+++			+		
Talaromyces wortmanni (Klöcker) C. R. Benjamin		++					
Talaromyces sp.		++					
Thielavia sepedonium Emmons		++	+		+		
T. terricola (Gilman & Abbott) Emmons		++	+		+		
Deuteromycetes							
Acremonium strictum W. Gams		++			+		
Acremonium sp.		++			+		
Acrophialophora fusispora (Saksena) M. B. Ellis		+++			++		
Alternaria humicola Oudem.		++++			+++		
A. alternata (Fr.) Keissler		++++			+++		
Aspergillus candidus Link		++++	++	+	++	+	
A. carbonarius (Bain.) Thom		+++++			+++		
A. carneus (v. Tiegh.) Blochwitz		++++			+++		
A. flavus Link		++++	+++	++	+++	++	+
A. fumigatus Fres.		+++++	+++	++	++++	++	+
A. fumigatus var. *acolumnaris* Rai, Agarwal & Tewari		++++	++++	++	++++	+++	+

A. fumigatus var. *albus* Rai, Agarwal & Tewari	+++++	++++	++	++++	++	+
A. fumigatus var. I (Unidentified)	+++++	+++	++	+++	++	+
A. japonicus Saito	+++++	+++	+	++++	++	
A. niger van Tiegh.	+++++	+++	++	++++	++	
A. niveus Blochwitz	++	+++	++++	+	++	+++
A. ochraceus Wilhelm	++++			++		
A. penicilliformis Kamyschko	++++			+++		
A. tamarii Kita	++++	++		++		
A. terreus Thom	+++++	++++	+++	++++	+++	++
A. terreus var. *africanus* Fennell & Raper	+++++	+++	++	++++	++	
A. ustus (Bain.) Thom & Church	+++++			+++		
Cladosporium cladosporioides (Fres.) de Vries	+++			++		
C. herbarum (Pers.) Link ex. Fr.	+++			++		
Curvularia lunata var. *aeria* (Batista, Lima & Vasconcelos) M. B. Ellis	+++++	+++		++++	+	
C. tetramera (Mc. Kinney) Boedijn ex Gilm.	++++			+++		
Curvularia sp.	+++++	+++		++++	++	
Drechslera spicifera (Bain.) v. Arx	+					
Doratomyces microsporus (Sacc.) Morton & Smith	+++			++		
Fungi Imperfecti (unidentified)	7	4	2	4	2	
Fusarium dimerum Penzig	+++			+++		
F. oxysporum Schlecht.	+++++	+++		+++	+	
F. roseum Link	++++			+++		
F. solani (Mart.) Sacc.	+++++	+++		++++	++	
Fusarium sp.	++++			+++		
Humicola fusco-atra Traaen	+++			++		
H. grisea Traaen	+++			+		
Humicola sp.	+++			++		
Paecilomyces variotii Bain.	+++			++		
Penicillium chrysogenum Thom	++++			+++		
P. citrinum Thom	++++	++		++++	+	
P. funiculosum Thom	++++			+++		
P. vinaceum Gilman & Abbott	++++			++		
Penicillium sp. I (Unidentified)	+++++	+++		++++	++	
Penicillium sp. II (Unidentified)	++++			+++		
Pestalotiopsis versicolor (Speg.) Steyaert	++++			+++		
Pestalotiopsis sp.	+++++	++		++++	+	
Rhizoctonia sp.	+++			++		
Scopulariopsis brevicaulis (Sacc.) Bain.	++			+		
Scopulariopsis sp.	++					
Trichoderma album Preuss	+++++	+++		+++	+	
T. koningii Oudem.	++++			+++		
T. lignorum (Tode) Harz	+++++	+++		++++	+	
Mycelia Sterilia	6	6	6	6	4	4

Table 2. Average percentage weight looses in wood as caused by some fungi

Test fungi	Average weight loss (in percentages)			
	Incubation period in days			
	15	30	45	60
Control	2.82	4.32	5.82	6.25
*Achaetomium luteum**	10.14	20.53	34.57	40.26
*Acremonium strictum**	6.84	13.32	22.71	25.25
Acremonium sp.*	6.83	13.66	23.41	29.89
*Alternaria alternata**	6.98	14.57	27.13	34.90
*Aspergillus fumigatus**	6.03	11.88	22.26	27.85
*A. terreus**	7.83	16.21	27.83	34.57
*A. terreus***	5.00	8.85	14.15	17.11
*A. ustus**	7.01	14.13	21.59	23.25
*Chaetomium globosum**	11.82	24.21	38.83	46.27
*Cladosporium herbarum**	8.37	14.73	23.82	29.13
Curvularia lunata var. *aeria**	10.84	21.22	34.84	43.46
Emericella nidulans var. *lata**	6.33	12.69	21.79	24.28
E. nidulans var. *lata***	4.03	8.33	13.77	16.44
*Fusarium oxysporum**	9.71	20.61	34.07	41.56
Humicola sp.*	11.78	23.53	36.62	43.14
*Microascus manginii**	8.48	17.49	29.66	39.21
*Penicillium funiculosum**	9.90	19.33	29.87	34.46
*Pestalotiopsis versicolor**	7.63	14.63	25.65	32.29
*Scopulariopsis brevicaulis**	5.21	10.15	17.88	21.05
Sporotrichum sp.*	6.71	12.34	21.20	24.74
Talaromyces sp.*	9.77	18.53	29.73	33.12
*Trichoderma lignorum**	10.82	21.61	34.58	42.26

* Mesophilic strain.

** Thermotolerant strain (Apinis, 1963; Evans, 1971).

rather rare. Of the Ascomycetes, *Chaetomium* spp. were more frequently isolated than the species of other genera (Rai and Chowdhery, 1978). The mesophilic forms were frequently found in wood samples that had decomposed to the greatest or least extent. From moderately decomposed wood samples, mostly thermotolerant forms, along with a few mesophilic forms were isolated.

The percentage weight losses for fungi tested on different days are given in Table 2, and have been classified into three groups based on weight loss——above 37% (strong), between 28 and 37% (moderate) and below 27% (weak). *Chaetomium globosum* had the greatest weight loss percentage (46.27), whereas *Scopulariopsis brevicaulis* had the least (21.05).

It is evident from Table 2 that in all cases the rate of weight loss increases up to 45 days, and decreases thereafter. It was also observed that at 29 ± 1 C, the thermotolerant strain of *Emericella nidulans* var. *lata* and *A. terreus* show less weight loss than mesophilic strain at the same temperature (Table 2).

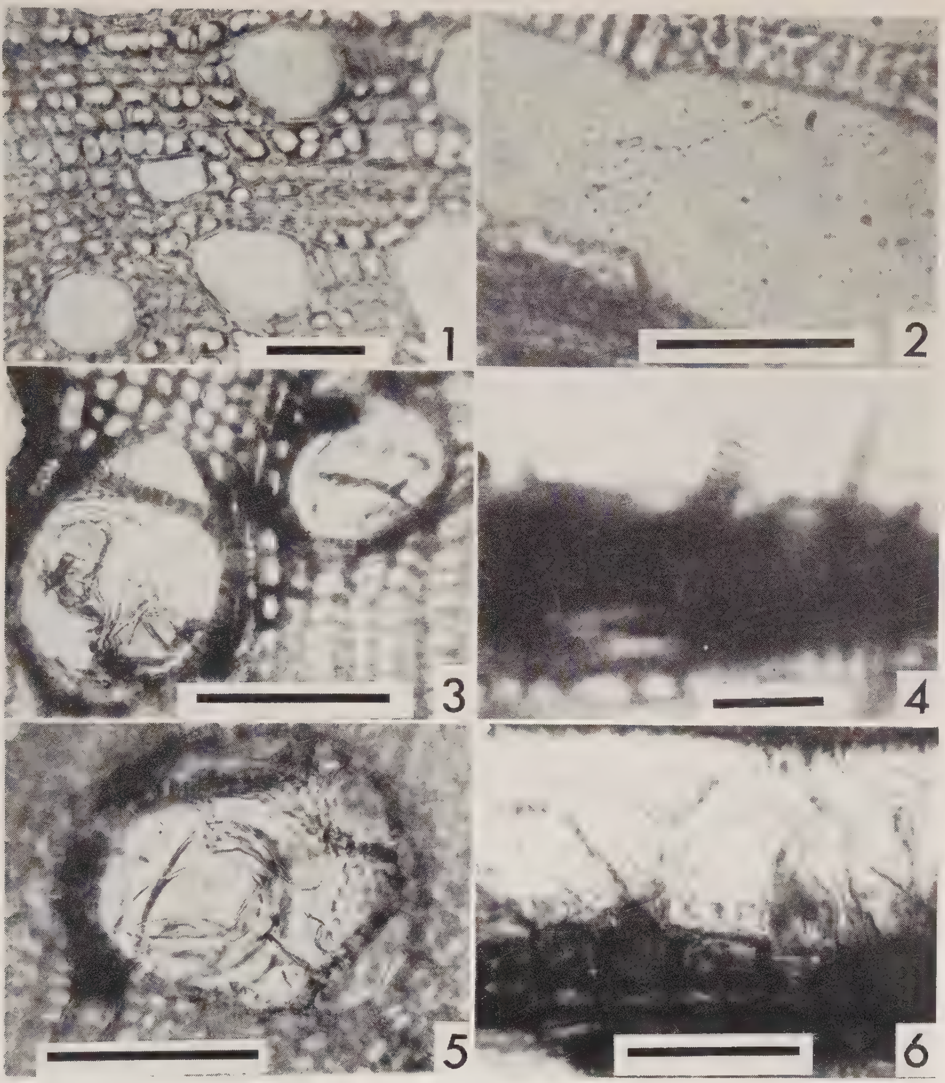


Fig. 2. Photomicrographs of wood blocks infested with fungi.

1. A transverse section of a control wood block (*Tamarindus indica*), showing uninfected cells and vessels. 2. A longitudinal section of wood (after 30 days) inoculated with *Penicillium funiculosum* showing fungal hyphae and conidia. 3. A transverse section of wood (after 45 days), showing hyphae of *Trichoderma lignorum* in vessels and neighbouring cells. 4. A longitudinal section of wood (after 60 days), showing conidium and mycelium of *Alternaria alternata*. 5. A transverse section of wood (after 45 days), showing infection by *Humicola* sp. into vessels and neighbouring cells. 6. A longitudinal section of wood (after 60 days), inoculated with *Chaetomium globosum*, showing fungal hyphae in tracheids and vessels. (Scales: 1 = 45 μm, 2 = 100 μm, 3 = 80 μm, 4 = 60 μm, 5 = 100 μm, and 6 = 95 μm.)

In transverse and longitudinal sections, a build-up of fungal mycelia was detected in ray parenchyma cells, tracheids and vessels. The cell walls of infected tracheids generally located in the neighbourhood of infected ray tissues, were also attacked (Fig. 2).

Discussion

The appearance of large number of fungal colonies and their higher growth rates on a malt extract agar medium are due to the medium's acidic pH which facilitates fungal growth and at the same time inhibits the growth of other microorganisms, for example, bacteria and actinomycetes (Alexander, 1961).

The Fungi Imperfecti constitute the group in which Aspergilli are most prevalent. The dominance of Aspergilli in mangrove swamps indicates that they are forms more tolerant to salt. This is also evident from the results of isolation experiments on media with 3% or 6% NaCl, and agrees with the results of Rai and Chowdhery (1978). In the present investigation the absence of Basidiomycetes may be due to the high concentrations of salt and other extreme ecological conditions found in the swamps (Rai and Chowdhery, 1978). On the other hand the occurrence of Basidiomycetes in mangrove swamps has been reported (Kohlmeyer, 1969).

The infrequent occurrence of Ascomycetes is due to the high salt concentrations, supported by the results of this experiment. *Hemisartorya* spp. are members of the strongly salt-loving ascosporic type, and can be isolated only on media with relatively high salt concentrations (3%), with increasing occurrence rates as the salt in the medium increases (to 6%). The same is also true for *Aspergillus niveus*. *Hemisartorya* species were isolated from the wood found in mangrove swamps for the first time here. However, *Hemisartorya maritima* was previously reported by Rai and Chowdhery (1975) from the mud of mangrove swamps.

As a result of microbial attacks, complex organic constituents break up into simpler substances and in the process, energy is released. This energy is sufficient to raise the temperature of the decomposing substances, with the result that some of the mesophilic forms get eliminated and their place is taken by thermophilous forms (Apinis, 1963; Evans, 1971). Later on, when most of the organic substances are utilized, the temperature of the decomposing material falls again and provides a better environment for mesophilic fungi. That is why only mesophilic forms were isolated from the least and most decomposed specimens, whereas mesophilic and thermotolerant forms were isolated from specimens moderately decomposed. This view is supported by the work of Chang and Hudson (1967).

The decomposition of organic matter serves two functions for the microorganisms. It provides energy for growth and supplies carbon for the formation of new cell material. Most plant material is composed of cellulose (up to 60%) and the maximum weight loss in woody material results from the disappearance of cellulose (Alexander, 1961). The highest percentage weight loss in the so-called strong group may be due to the greater level of activity of cellulase and other wood destroying enzymes, and lower weight losses to comparatively lower levels of enzyme activity. The higher cellulolytic activity of

fungi, classified here as the strong group, has been reported by several workers (Greathouse and Ames, 1945; Basu, 1946; White et al., 1948).

Fungi tested here caused maximum weight losses during the first 45 days of incubation. Subsequent decreases in the rate of weight loss may be the result of the production of toxic by-products during rapid fungal growth which in turn may be inhibitory to further growth.

Smaller weight losses in thermotolerant strains at 29 ± 1 C may be due to the effects of temperature, because the above temperature is not suitable for the optimal growth. Rather, the optimum temperature for such fungi ranges between 35 and 40 C (Evans, 1971).

The microscopic examination of sections shows the presence of fungal hyphae in parenchyma cells, tracheids and vessels. It seems that hyphae first penetrate the thin-walled parenchyma cells, and then the tracheids and vessels. Penetration of wood blocks by hyphae also took place from transverse surfaces directly into vertical elements.

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Iron acetocarmine for nuclear staining in rust fungi

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小　野　義　隆*：鉄酢酸カーミンによるさび菌の核染色

Summary

Belling's iron acetocarmine was applied to stain the nuclei of rust fungi. This is an easy and rapid method which allows the clear differentiation of nuclei of rust spores and basidia from both fresh and dried herbarium materials. Very poor results, however, were obtained using rust spores with thick or dark-pigmented walls.

Various staining techniques have been developed to differentiate nuclei in spores, germ tubes, basidia, and hyphal cells of rust fungi. Among these, Heidenhain's iron-alum hematoxylin with various modifications (Schneider, 1973), HCl-Giemsa described by Colotelo and Grinchenko (1962), and acetocarmine with a mordant of iron-alum (Singh and Pavgi, 1966) have been widely employed in recent years. However, these techniques are restricted to fresh materials, and require rather cumbersome procedures such as fixation, dehydration and rehydration, hydrolysis, and bleaching. The author found that iron acetocarmine, originally designed by Belling (1926) and redescribed by Schneider (1973), was a suitable method to stain rust fungus nuclei of both fresh materials and dried herbarium specimens, because of its simplicity and speed.

Materials and Methods

Rusts examined Rust species and kinds of spores examined were randomly selected, and included:

Aeciospores of *Cerotelium dicentrae* Mains & Anderson on *Dicentra cucullaria* (L.) Berhn., Tippecanoe Co., Ind., U. S. A., 12 May 1978, Y. Ono (US-303); and *Puccinia podophylli* Schw. on *Podophyllum peltatum* L., Tippecanoe Co., Ind., U. S. A., 15 April 1978, Y. Ono (US-243). Urediniospores of *Melampsoridium alni* (Thuem.) Diet. on *Alnus sieboldiana* Matsum., Unomisaki, Taga-gun, Ibaraki, 8 Oct. 1980, Y. Ono (no. 546); *Melampsora* sp. on *Salix* sp., Unomisaki, Taga-gun, Ibaraki, 8 Oct. 1980, Y. Ono (no. 542); *Coleosporium asterum* (Diet.) Syd. on *Solidago altissima* L., Onuki, Higashiibaraki-gun, Ibaraki, 29 Oct. 1980, Y. Ono (no. 554); *Coleosporium clematidis* Barcl. on *Clematis paniculata* Thunb., Uno-

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misaki, Taga-gun, Ibaraki, 8 Oct. 1980, Y. Ono (no. 544); *Cerotelium dicentrae* Mains & Anderson on *Laportea canadensis* Gaud., Tippecanoe Co., Ind., U. S. A., 2 July 1977, Y. Ono (US-37); *Phakopsora pachyrhizi* Syd. on *Glycine max* (L.) Merr., Mito, Ibaraki, 24 Oct. 1980, Y. Ono (no. 551); *Kuehneola uredinis* (Link) Arth. on *Rubus* sp., Fannin Co., Ga., U. S. A., 23 Aug. 1977, J. F. Hennen, J. W. McCain & Y. Ono (US-171); and *Uredo iyoensis* Hiratsuka, f. & Yoshinaga on *Viola vaginata* Maxim., Yonezawa, Yamagata, 30 Sept. 1973, Y. Ono (no. 86). Amphispores of *Uredinopsis pteridis* Diet. & Holw. on *Pteridium* sp., Oconto Co., Wis., U. S. A., 13 Aug. 1977, Y. Ono (US-106); *Hyalopsora polypodii* (Diet.) Magn. on *Cystopteris fragilis* Berhn., Tippecanoe Co., Ind., U. S. A., 17 Sept. 1977, Y. Ono (US-235). Teliospores of *Uredinopsis pteridis* Diet. & Holw. on *Pteridium* sp., Fannin Co., Ga., U. S. A., 23 Aug. 1977, J. F. Hennen, J. W. McCain & Y. Ono (US-173). Basidia of *Kuehneola uredinis* (Link) Arth. on *Rubus* sp., Fannin Co., Ga., U. S. A., 23 Aug. 1977, J. F. Hennen, J. W. McCain & Y. Ono (US-171); and *Puccinia elytrariae* P. Henn. on *Justicia procumbens* L., Mito, Ibaraki, 23 Sept. 1980, Y. Ono (no. 529). Basidiospores of *Puccinia elytrariae* P. Henn. on *Justicia procumbens* L., Mito, Ibaraki, 23 Sept. 1980, Y. Ono (no. 529).

Preparation of iron acetocarmine Iron acetocarmine was prepared as the procedure described by Schneider (1973) (Fig. 1). Two grams of carmine powder (Merck) were mixed in 100 ml of hot 45% acetic acid, and boiled for 2 min. The mixture was cooled at room temperature and filtrated. To one half of this liquid, a concentrated acetic solution of ferric hydroxide was added until the liquid became slightly bluish-red, but did not form a visible precipitate. Subsequently, it was mixed with the remaining half of the untreated acetocarmine. Iron acetocarmine, thus prepared, remained effective for

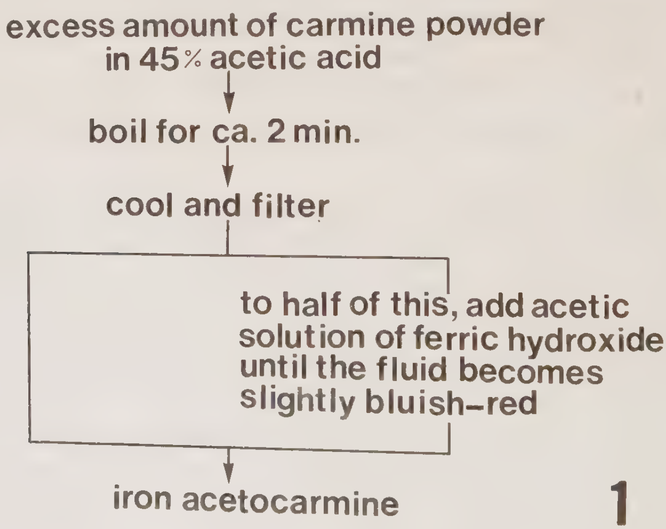


Fig. 1. Preparation of iron acetocarmine (after Schneider, 1973).

about a month in a brown vial at room temperature, and for a few months at 2–4 C. Ferric hydroxide was precipitated from the ferric chloride solution by the addition of a slight excess of sodium hydroxide. The precipitate was throughly washed with distilled water, air-dried, and preserved at 2–4 C until it was to be used.

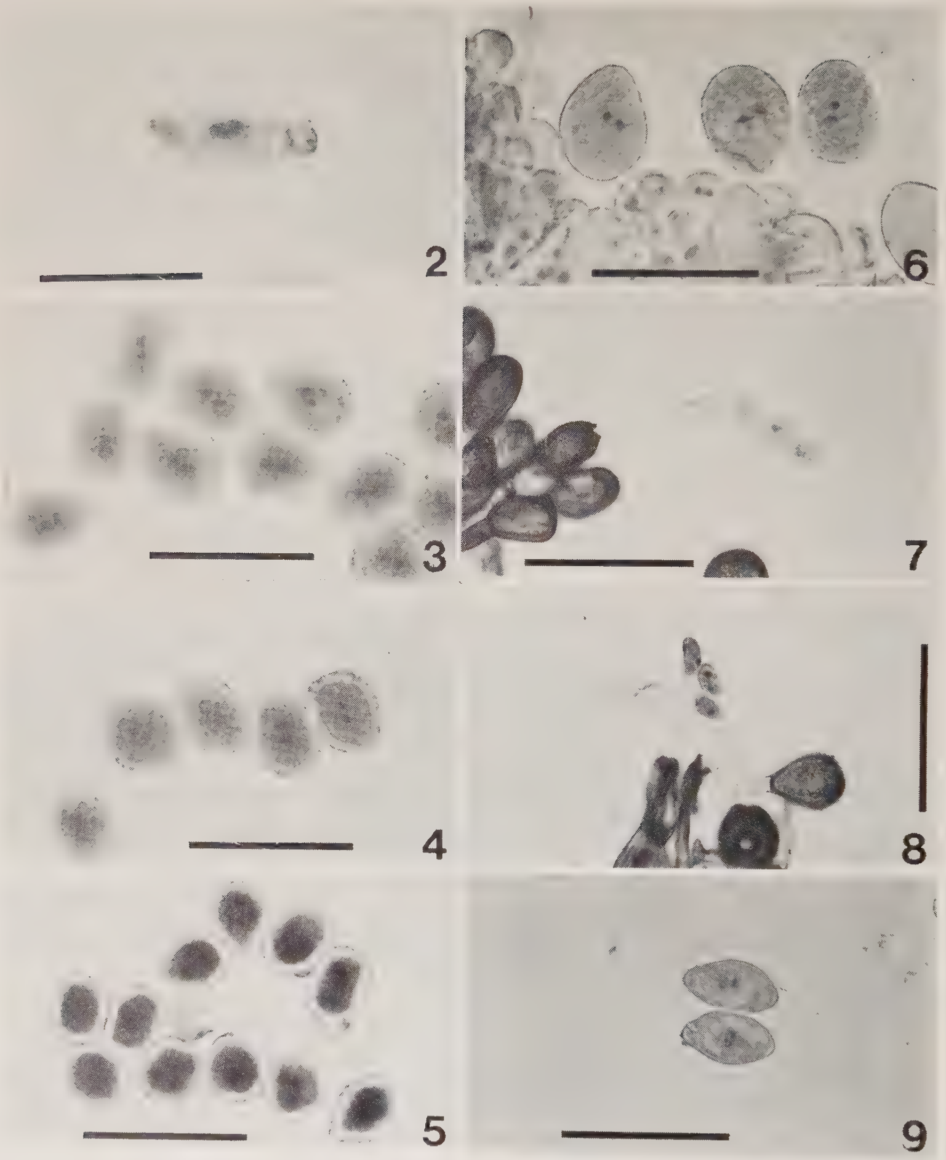
Staining procedures Spores were scraped from sori, and mounted directly in a drop of the iron acetocarmine fluid on glass slides. The slides were gently heated, until just prior to the boiling level to increase the intensity of the color of stained nuclei. Free-hand sections of sori were also stained in the same manner. In order to stain fresh basidia and basidiospores, teliospores were germinated on a cover slip in a moist chamber, and the slip was put on a drop of iron acetocarmine on a slide. These slides were sealed with two strokes of fingernail polish to prevent the liquid from drying up, and observed within one or two days because the preparations lack permanency.

Results and Discussion

Figures 2–8 show the stained spores and basidia from fresh materials, and Figs. 9–17, those from herbarium specimens. Nuclei were stained translucent red, purple to dark red, and the cytoplasm was unstained or pale red. Stained nuclei were seen more clearly when observed through a green filter under a blight-field microscope, nuclei appearing much darker or almost black. Nuclei in thick-walled spores tended to stain more slowly than those in thin-walled spores, the former required 5–20 min whereas the latter stained immediately after heating. Generally, nuclei of aecidioid verrucose spores, e.g. aeciospores of *Puccinia* and *Cerotelium* and urediniospores of *Coleosporium*, were more rapidly and better differentiated than those of uredoid echinulate spores, e.g. urediniospores of *Melampsoridium* and *Melampsora*. This is probably due to the varying permeabilities of spore walls. Nuclei became progressively darker and intense in color. The stained nuclei, however, became indistinguishable with time because the cytoplasm also darkened progressively.

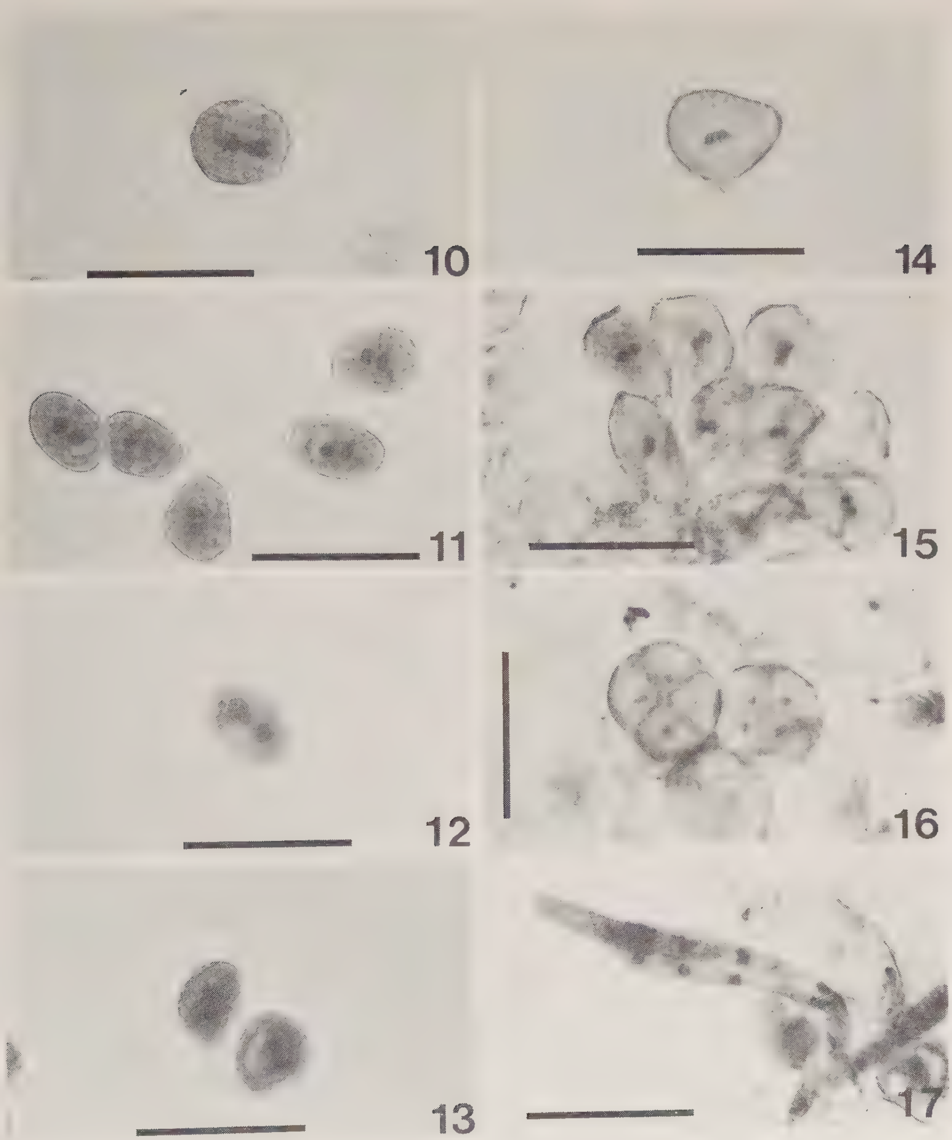
Nuclei in the spores and basidia of herbarium specimens were somewhat better differentiated than those from fresh materials (Figs. 9 17). Despite the fact that cytoplasmic contents had almost completely decomposed, nuclear substances seemed to preseve well for a considerable length of time kept in dry conditions. The author was able to obtain reasonably good results from herbarium specimens several years old. Cunningham (1968) reported a paraffin-sectioning method for herbarium specimens and stained nuclei in teliospores of these specimens. This is the only previously reported attempt to stain nuclei of spores of herbarium specimens.

Staining with iron acetocarmine can be used effectively to survey the nuclear cycle and its behavior in rust fungi even in herbarium specimens whose fresh materials are not easily obtained. This staining technique gave only poor results, however, for rust spores with thick or dark-pigmented walls, e.g. uredinio- and teliospores of many *Puccinia* species.



Figs. 2–9. Iron acetocarmine staining of rust spores and basidia. Nuclei appear black, circular or as slightly irregular dots in the spores or basidia. Bars in all figures represent 50 μm.

2. Urediniospore of *Melampsoridium alni* (no. 546). 3. Urediniospores of *Coleosporium asterum* (no. 554). 4. Urediniospores of *Coleosporium clematidis* (no. 544). 5. Urediniospores of *Melampsora* sp. (no. 542). 6. Urediniospores of *Phakopsora pachyrhizi* (no. 551), in a vertical section of uredinium. 7. Basidium of *Puccinia elytrariae* (no. 529). One nucleus is present in each basidial cell. 8. Basidiospores and a collapsed basidium of *Puccinia elytrariae* (no. 529). Each basidiospore contains two nuclei, which seem to have divided from the nucleus in a basidial cell just after translocation. 9. Urediniospores of *Uredo iyoensis* (no. 86).



Figs. 10–17. Iron acetocarmine staining of rust spores and basidia. Nuclei appear black, circular or as slightly irregular dots in the spores or basidia. Bars in all figures represent 50 μm.

10. Aeciospores of *Puccinia podophylli* (US-243). 11. Aeciospores of *Cerotelium dicentrae* (US-303). 12. Urediniospores of *Cerotelium dicentrae* (US-37). 13. Urediniospores of *Kuehneola uredinis* (US-171). 14. Amphispores of *Hyalopsora polypodii* (US-235). 15. Amphispores of *Uredinopsis pteridis* (US-106), in a vertical section of a sorus. 16. Teliospores of *Uredinopsis pteridis* (US-173). 17. Basidia and collapsed teliospores of *Kuehneola uredinis* (US-171). Each basidial cell contains one nucleus.

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摘　要

Belling の鉄酢酸カーミンをさび菌の核染色に応用した．この染色液による方法は，簡単かつ迅速で，新鮮な材料のみならず乾燥標本からのさび胞子，夏胞子，担子器および担子胞子，比較的膜の薄い冬胞子などの核染色に良好な結果をもたらした．しかし，強く着色した厚膜の冬胞子などでは，良い結果が得られなかった．

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Taxonomic studies on Indian Myxomycetes II. The development of cortical scales in *Lycogala* species and their bearing on the delimitation of species

T. N. Lakhanpal* and K. G. Mukerji**

Summary

Three trends in the development of cortical scales are traced in the genus *Lycogala*. Smooth and circular or irregular scales which represent the basic type, are found in *L. epidendrum*. These basic scales on one hand, give rise to reticulate scales of *L. conicum* by branching and ramification without cleavage, and on the other hand, produce tessellated scales of *L. exiguum* (including *L. epidendrum* var. *tessellatum*) by internal cleavage into vesicles or 'cells'.

The delimitation of the species of *Lycogala* became fairly clear with Martin's (1967) comments on the controversial *L. exiguum* and *L. epidendrum* var. *tessellatum*. Nannenga-Bremekamp (personal communication, 1974), however, upheld *L. epidendrum* var. *tessellatum*. The development of cortical scales in *L. epidendrum* (L.) Fr., *L. conicum* Pers., *L. exiguum* Morgan, and *L. epidendrum* var. *tessellatum* is discussed in this paper.

The cortex of developing and fully formed fruiting bodies was fixed and mounted in Hoyer's medium containing a few drops of cotton blue. Observations of the cortex-mounts made from young and immature aethalia revealed single, smooth and circular type scales, whereas the mounts from nearly ripe fruiting bodies, showed three different types of scales for the three different species, all of which developed from the common smooth and circular type. It is interesting to note that all stages in the development of each of these three types could be traced in cortical mounts from a single, mature aethalium.

In *L. exiguum* and *L. epidendrum* var. *tessellatum* the scales are enlarged and divided internally into compartments or 'cells' giving a tessellated appearance. In *L. epidendrum* and *L. conicum*, however, the enlargement of scales was not accompanied by their internal segmentation. The arrangement of scales in these two species is quite distinct at maturity. In *L. conicum*, the scales are elongated and anastomosed intricately into a network or reticulum. In contrast with this, the scales of *L. epidendrum* are simply elongated, enlarged and swollen without much overlapping.

Lister (1925) and Hagelstein (1944) reduced *L. exiguum* to a variety of *L. epidendrum*, maintaining var. *tessellatum* as a distinct taxon. They characterized *L. epidendrum* var.

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Table 1. Comparison of morphological characteristics of *Lycogala* species

Name of species	*Lycogala epidendrum*	*L. epidendrum* var. *tessellatum*	*L. epiguum*	*L. conicum*
Specimen(s) No.	TNL/180, 181	TNL/497	TNL/132, 167, 168	TNL/489, 492, 738
Fructifications: size and characteristics of cortex	2–10 mm: dark reddish brown or almost black: cortical scales circular or irregular, flattened unbra-nched and uncleaved	3–8 mm; dark brown; cortical scales pustullate and tessellate	2–5 mm; smooth or minutely roughened; cortical scales pustullate and tessellate; scale development of cleavage type	2–3(–8) mm; dark brown or almost black; cortical scales pustullate, branched and ramified intricately; scale development of non-cleavage type
Pseudocapillitium: diam and markings	Threads 10 20 μm, faintly or prominently wrinkled	Treads 5–10 μm, very prominently wrinkled	Treads 2–5(–7) μm, prominently wrinkled	Threads 5–11 μm, very prominently wrinkled
Spores: size in diam and markings	6–7.5 μm, prominently reticulate over 3/4 surface	6 μm, prominently reticulate over 7/8 spore surface	5–6.5 μm, faintly reticulate over 3/4 spore surface	5–6(7.5) μm, reticulate over 3/4 spore surface
Specimen(s) No.	TNL/288	TNL/490	TNL/493	TNL/494
Fructifications: size and characteristics of cortex	3–6 mm; smooth, brownish-black; only cortical scales pustullate; scales development of non-cleavage type	4–5 mm; black, cortical scales smooth, circular or irregularly pustullate; scales development non-cleavage type	2–4 mm; brown; cortical scales form a prominent reticulum on the cortex externally, in mounts smooth, circular or irregularly pustullate; scale development of non-cleavage type	1–4 mm; brown; cortical scales forming a honey coloured reticulum externally on the cortex, internally circular or irregular, pustullate; scale development of non-cleavage type

Pseudocapillitium: diam and markings	10–11(–12) μm, wrinkles deep and prominent	7 μm, wrinkled	7–10 μm, wrinkled	4.5–7.5 μm, wrinkled
Spores: size in diam and markings	4.5–6 μm, prominently reticulate over 3/4 spore surface	6 μm, reticulate over 7/8 spore surface	6 μm, spore reticulate over 7/8 surface	6 μm, prominently reticulate over 3/4 spore surfcae
Specimen(s) No.	TNL/495	TNL/496	TNL/498	TNL/499
Fructifications: size and characteristics of cortex	1–3 mm; dark brown; scales formming an apparent reticulum externally, smooth pustullate or irregular under the microscope; scale development of non-cleavage type	3–4 μm; brown; scales honey coloured, smooth pustullate or irregular; scale development of non-cleave type	1–4 mm; reddish brown; scale honey coloured, forming an apparent reticulum, simplė, circular and non-tessellate under the microscope; scale development of non-cleavage type	2–6 mm; brownish black; scales forming an apparent reticulum externally, smooth, circular or irregular under the microscope; scale development of non-cleavage type
Pseudocapillitium: diam and markings	6–7.5 μm, faintly wrinkled	10 μm, wrinkled	up to 10 μm, prominently wrinkled	below 10 μm, prominently wrinkled
Spores: size in diam and markings	6 μm, prominently reticulate over 3/4 spore surface	6 μm, faintly reticulate over 7/8 spore surface	6 μm prominently reticulate over 7/8 spore surface	6–7.5 μm prominently reticulate over 7/8 spore surface

exiguum by small (2–5 mm) dark aethalia without chambered vesicles, and the latter by tessellated scales. Nannenga-Bremekamp (1962) maintained *L. epidendrum* var. *tessellatum* for populations with a tessellated cortex composed of 20–70 μm wide 'cells'. She expressed doubts concerning the existence of *L. exiguum* in the Netherlands, and separated it from *L. epidendrum* var. *tessellatum* on the basis of smaller fructifications, smooth capillitium, and spores. Martin (1967) recognized *L. exiguum* as a distinct species, as was the case with Morgan (1893) and Macbride (1899; 1922), but treated *L. epidendrum* var. *tessellatum* as its synonym. He reduced *L. epidendrum* var. *tessellatum* to *L. exiguum* because of the existence of several intergrades showing complete gradations from pustullate to tesselate scales. He distinguished *L. exiguum* from *L. epidendrum* by its smaller size (1–3 mm) pustullate and tessellate cortical scales, narrower (less than 10 μm) and smooth capillitium, and finely reticulate or smoother and smaller (5.5 μm across) spores.

Among the different collections made from Himachal Pradesh (abbr. H.P.), India, TNL*/497 is referable to var. *tessellatum*, TNL/132, 167 and 168 are similar to *L. exiguum*, and TNL/180 and 182 resemble *L. epidendrum*, whereas TNL/489, 492, 738 and others sent by Mrs. Nannenga-Bremekamp belong to *L. conicum*. The remaining collections TNL/288, 491, 493, 494, 495 and 499 are neither referable to var. *tessellatum* nor to any of the these three species. The characters of all populations are given in the Table 1.

It is evident from the table that populations TNL/288, 491, 493, 494, 495, 496, 498 and 499 are characterised by pustullate or pustullate-irregular whole scales, sometimes branched and ramified, minutely or strongly wrinkled pseudocapillitium, mostly under 10 μm in diam. The spores are delicately but prominently reticulate for over seven-eighths of the spore surface and 5–6(–7.5) μm in diam. These resemble *L. epidendrum* in the nature of their fructifications, size and spore ornamentation, and in the nature of the pseudocapillitium and spores. They are similar to *L. exiguum* in terms of spore size and pseudocapillitial diameter. Characteristics are so variable among these collections that it is virtually impossible to classify them, and this suggests that *L. epidendrum*, *L. exiguum* and *L. epidendrum* var. *tessellatum* (sensu lato) may in fact constitute a species complex. These variations, in fact, led Martin (1967) to treat *L. epidendrum* var. *tessellatum* as a synonym of *L. exiguum*.

The study of variation in the morphology of scales suggests that there are three developmental trends from a smooth and circular type. One trend involves the cleavage of pustullate scales leading to the formation of tessellate scales as exemplified by *L. exiguum* (Fig. 2 m and o), and *L. epidendrum* var. *tessellatum* (Figs. 1, 2 j–m). The second involves the swelling, enlargement and flattening of the scales or pustules leading to the direct formation of non-tessellate scales, as exemplified by *L. epidendrum* (Fig. 1 a–f). In the third case, the pustules swell, enlarge, branch and anastomose (without cleavage) forming an

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intricate net or reticulum. This is exemplified by *L. conicum* (Fig. 1 g and i). It may be pertinent to mention here that cortical scales are totally absent in *L. flavofuscum*.

Although characters, such as the size of fructifications and the size and ornamentation of pseudocapillitium and spores, that have been used for the delimitation of these three highly plastic taxa by earlier taxonomists, are highly variable, the scale development is so constant that one can group these populations into distinct species on this criterion alone – *L. exiguum* (including *L. epidendrum* var. *tessellatum*) which is characterized by a cleavage type of scale development, and *L. epidendrum* and *L. conicum* which are recognizable by their non-cleavage type of scale development (Fig. 3).

Martin (1967) seems to be correct in treating *L. epidendrum* var. *tessellatum* as a synonym of *L. exiguum*, as the former shows cleavage scale development, the diagnostic feature

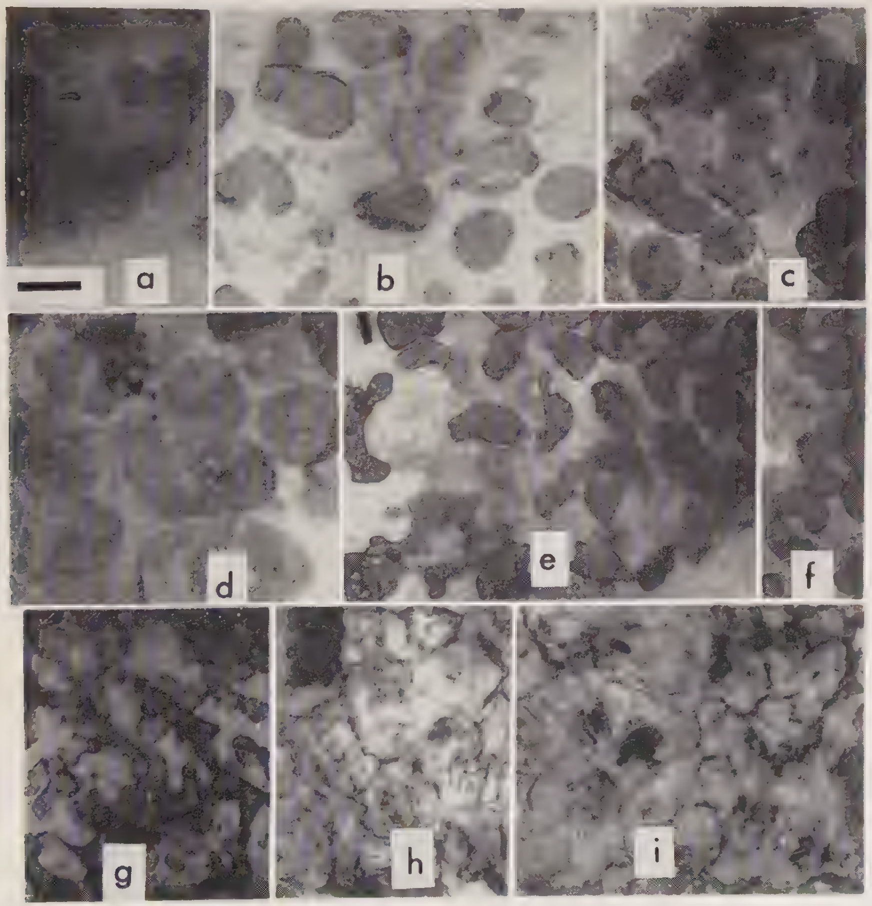


Fig. 1. *Lycogala epidendrum* (L.) Fr. (a–f) and *L. conicum* Pers. (g–i). a. Circular and pustullate cortical scales. b–f. Pustullate scales dividing without cleavage into irregular whole scales. g–i. Simple pustullate scales branching without cleavage to form an intricate reticulum. (Bar denotes 10 μm; scales for b–i = a)

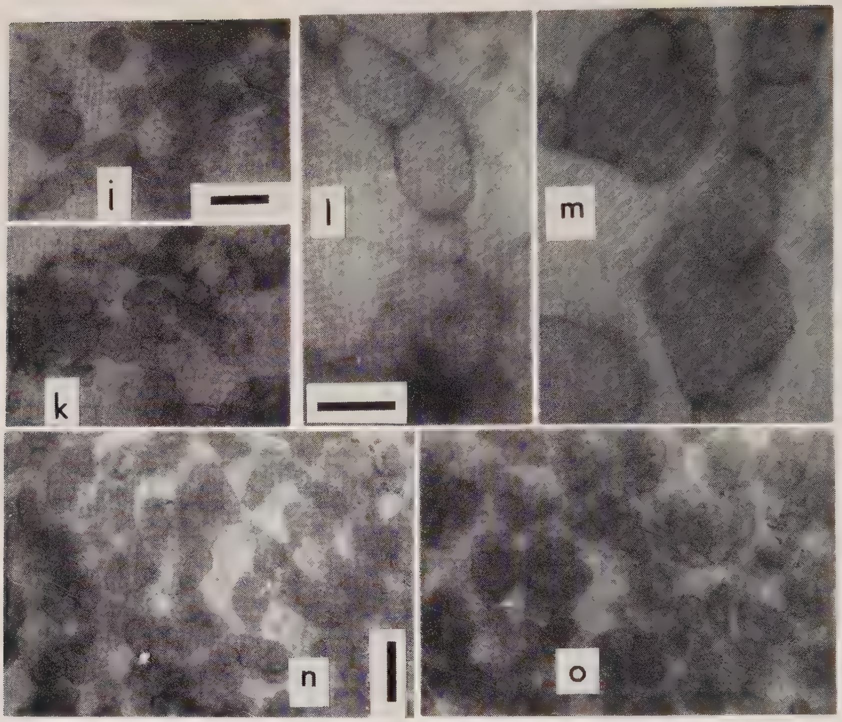


Fig. 2. *Lycogala epidendrum* var. *tessellatum* A. Lister (j–m) and *L. exiguum* Morgan (n–o). j–k. Simple and cleaved pustullate scales becoming tessellated. l–m. Enlarged scales from j and k. n. Simple scales cleaved into 2,3 and more 'cells'. o. Tessellated scales. (All bars denote 10 μm; scale for k = i, scale for m = l, and scale for o = n)

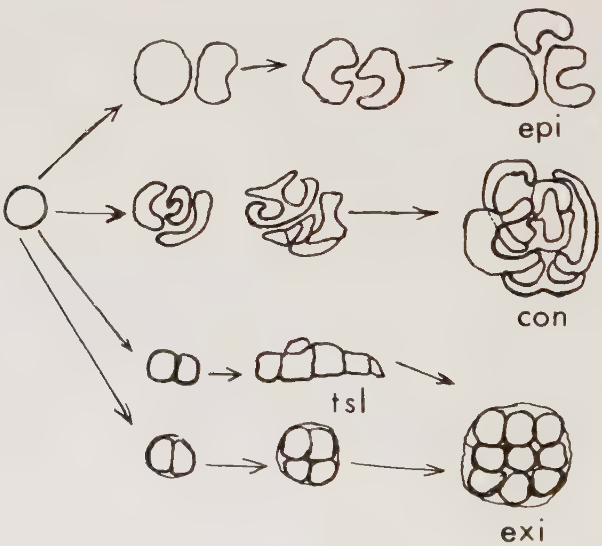


Fig. 3. Diagrammatic sketch showing the probable ontogeny of cortical scales in *Lycogala epidendrum* (epi), *L. conicum* (con), *L. epidendrum* var. *tessellatum* (tsl) and *L. exiguum* (exi) from a simple, pustullate scales.

L. exiguum. Nannenga-Bremekamp's treatment of the variety *tessellatum* as a distinct taxon on the basis of the size of the vesicles is not fully justified, as the size ranges of the vesicles frequently overlap. Therefore, the var. *tessellatum* should be treated as a synonym of *L. exiguum*, as by Martin did.

The authors gratefully acknowledge the assistance of Professors H. Y. Mohan Ram and B. M. Johri for providing experimental facilities, Mrs. N. E. Nannenga-Bremekamp for the exchange and authentication of the specimens and Professor C. J. Alexopoulos and Dr. C. R. Babu for their critical reading of the manuscript.

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新 刊 紹 介

Arx, J. A. von: The Genera of Fungi Sporulating in Pure Culture. 3rd ed. 424 p., 99 figs. J. Cramer—A. R. Cantner, FL-9490 Vaduz. 1981. DM 120.

本書は初版（1970年発行）以来，培養できるカビ・酵母について，属を検索しようとするとき，大変便利な手引きとして好評であった．1974年第2版（cf 日菌報 **16**(2)：102．1975）はわずかな改訂にとどまったが，今回の第3版はかなり大改訂である．装丁や体裁，紙質など全く第2版と同じであるが，内容が増加し分厚くなっている．図版の数は整理の結果，むしろ減少しているが，プレート1頁あたりの収容数がふえたため，全体としては約70属以上もの形態が新らたに図示されているという．もちろん，前版の図をそのまま再録もされているが，丁寧にみると，修正加筆して，よりよい図に替えられている場合も少なくない（例：p. 73, *Cephaloascus fragrans*）．

初版が刊行された当時（1970年）の状況と比べ，最近では接合菌類や不完全菌類を扱った検索のための類書が，他の研究者によって次々と出版され，その内容も大変すぐれている．本書がはじめて菌類の分類・同定に利用されたときに果した役割は高く評価されるべきであるが，卒直にいって現在では本書の利用価値がもっとも高い部分は，子のう菌類の項目である．この際，改訂は本版かぎりで思い切った転換を図る時期にきているように印象づけられた．内容の一部について多少の評言を以下に述べる．

前版で取り扱いが中途半端であった *Cryptococcus* ほかの担子菌系酵母菌類については，今回整理されている．

Eurotiales も今回は科ごとにまとめ，Trichocomaceae, Onygenaceae (Gymnoascaceae), Pseudeurotiaceae...... としている．ただし，最後の項を十把一からげにされているのは預けない．

Eremascus が本文は88頁にあって，図 (Fig. 38) が139頁というのも不親切である．恐らく *Monascus* との関係から図を配置したのであろうが，*Monascus* の説明文にそのことを触れ，関係頁数を示すよう配慮されるべきである．

Sphaeriales の項では，大幅に図を改めており，また ostiole の有無によって区別されていた一部の属を整理している（例：*Melanospora* (syn. *Microthecium*), *Gelasinospora* (syn. *Anixiella*))．しかしながら，一方において *Europhium*—*Ceratocystis*, *Petriellidium*—*Petriella*, *Chaetomidium*—*Chaetomium*, *Echinopodospora*—*Apiosordaria*, ... などの関係をそのままにしているのは，一貫した姿勢とはいえず，検索にあたって読者の混乱を招くであろう．また，*Coniochaeta* およびその関連属は明らかに Xylariaceae に移すべきものであろう．

Sphaeriales は Xylariaceae, Hypocreaceae......など，もう少し細分して扱うべきではなかろうか．見解によっては，それぞれ別々の目に扱われる菌類であり，上記 Eurotiales を細分化したのに対し，あまりにもアンバランスである．

以上のように種々の問題点はあるが，1980年までの最新情報が要領よくまとめられていること，著者の見解が短かいスペースながらうかがえることは，teleomorph—anamorph の関係を探す場合とともに，本書が依然として利用価値のある存在であることを示すものである．

(Shun-ichi Udagawa　宇田川　俊一)

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エノキタケの育種
1. 検定系統による優良単核系統の選抜

李　　璧 如・衣 川 堅 二 郎*

Pi Ju LEE and Kenjiro KINUGAWA*: A breeding method for *Flammulina velutipes*.
1. Selection of monokaryotic strains by the use of testers

Summary

Flammulina velutipes (Japanese-"enokitake") was used as the subject in an attempt to determine a feasible breeding method.

Monokaryons with different fruiting potentials, ♯7-1, ♯7-2 and ♯5s5, isolated from wild Canadian strains, were used as test strains. Many synthetic dikaryons were produced by crossing the testers with the twenty-seven monokaryons originating from ♯11, a commercial Japanese strain. Next, the dikaryons were examined to determine fruiting ability, mycelial growth based on colony expansion, and certain morphological characteristics of the resulting fruit-body. The fruiting ability of synthetic dikaryons was found to be highly variable, and is apparently regulated by quantitative inheritance and heterosis. Component monokaryons of those dikaryons capable of producing plentiful fruit-bodies were found among those with colony expansion rates greater than 0.6 cm/day. The dikaryons tended to show relatively higher rates of production (mycelium and fruit-body dry weights) and greater fruiting efficiency rates (fruit-body dry weight/combined dry weight of mycelium and fruit-bodies). In the primary test of the performance of selected monokaryons, a synthetic dikaryon ♯11s24(×5s5) yielded greater number of fruit-bodies than the current commercial stock, although the fruit-bodies were of an unmarketable coloration.

In conclusion, the use of *Flammulina* tester strains at the start of breeding proved fairly efficient in sorting out monokaryotic strains useful for further breeding, and thus provided a saving of much labor over the conventional method of inter-mating monokaryons.

食用キノコの育種に関する興味ある報告として次のようなものがある．Eger(1974) は，ヒラタケ (*Pleurotus ostreatus*) に関し，単核性発茸（単核菌糸による子実体形成）の能力のある単胞子菌糸系統を選抜すれば，育種の労力と時間を節約することができると報告した．Esser et al. (1974) は *Agrocybe aegerita* を用い，複核菌糸が子実体発生に至るまでに必要な時間によって，親になっている単核菌糸体の発茸能力の大小を決めようとした．彼らは小さな自殖系集団の内部で，4 世代に亘る交配と選抜を行ない，発茸能力が始めより高い系統を得た．また，単核性発茸とそれによって構成された複核菌糸の発茸能力の間には統計上意味のある相関があると主張した．また，Esser and Stahl (1975) は同じ材料の複核菌糸体の発茸能力は単核性発茸を支配する遺伝子 (mf+) の存在によって促進されるようであるといっている．*Agaricus bisporus* を用いた育種例は比較的多い．Kneebone et al. (1977) は *Agaricus bisporus* の単胞子起原の菌糸

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体の選抜によって，現在アメリカで産業的によく栽培されている品種より生産量が高い系統を少なくとも12系統得ることができた．一方，Raper and Krongelb (1958) はスエヒロタケ (*Schizophyllum commune*) を用いて，発茸能力の遺伝に関する研究を行なった．彼らは，発茸能力はゲノムに分散している多くの遺伝子によって支配を受けていることと，単核菌糸の発茸能力は発茸能力の低い検定系統と交配することにより推定できることなどを示した．以上のように，担子菌の子実体形成は明らかに遺伝的支配を受けていて，有効な育種法を示唆する実験があるにもかかわらず，これまで食用菌一般に通用する育種方法を確立してきたとは考えられない．ここでは，より便利で効率的な食用キノコの育種法を見いだすことを目的として実験を行なった．

基本的な考えは，交配育種において膨大な労力を要する任意な交配法を用いず，素材となる複核菌糸の単胞子に由来する多くの単核系統を，あらかじめ選定した検定系統と交配し，その結果によって，望ましい単核系統を選抜するということである (cf. Raper and Krongelb, 1958; Esser et al., 1974)．本実験の結果は，以上の方法が実用的育種法としてかなり期待できることを示すものと考える．

材料および方法

材料：Table 1 に示す #11 は，育種の素材とする複核菌糸で，大阪府能勢町天王の能勢エ

Table 1. List of strains used

Accession number	Mating type	Characteristics*	Origin and remarks
#5s5	A_9B_9	a.h.++, m.d.++, m.f.+++	Monospore-(#5s5) and mono-oidium-(#7-1 and #7-2) cultures from #5 and #7, wild Canadian strains supplied by Dr. R. J. Bandoni (Univ. of British Columbia).
#7-1	A_6B_6	a.h.+, m.f.−	
#7-2	A_7B_7	a.h.−, m.f.+	
#11	$A_4B_4+A_5B_5$	a.h.++, m.d.++	A Japanese commercial strain, "Maruei", isolated from stipe tissue supplied by the Nose Enokitake Production Cooperative, Osaka.
#11s11	A_4B_4	a.h.++, m.f.+++	Monospore cultures from #11.
#11s22, 23, 25, 28, 30, 31	A_4B_4	a.h.+, m.f.−	Monospore cultures from #11.
#11s9	A_5B_5	a.h.++, m.f.−	Monospore cultures from #11.
#11s24	A_5B_5	a.h.++, m.f.+++	Monospore cultures from #11.
#11s7, 8, 27	A_5B_5	a.h.+, m.f.−	Monospore cultures from #11.
#11s14, 20	A_4B_5	a.h.++, m.f.−	Monospore cultures from #11.
#11s18, 19	A_4B_5	a.h.++, m.f.+	Monospore cultures from #11.
#11s1, 10, 16	A_4B_5	a.h.+, m.f.−	Monospore cultures from #11.
#11s12	A_4B_5	a.h.+, m.f.+	Monospore cultures from #11.
#11s2, 3, 5	A_5B_4	a.h.++, m.f.+	Monospore cultures from #11.
#11s4	A_5B_4	a.h.++, m.f.−	Monospore cultures from #11.
#11s13, 17	A_5B_4	a.h.+, m.f.−	Monospore cultures from #11.
#11s21	A_5B_4	a.h.+, m.f.+	Monospore cultures from #11.

* a.h., aerial hyphae; m.d., mycelial density; m.f., monokaryotic fruiting.
+++, abundant; ++, medium; +, infrequent; −, none.

ノキタケ生産組合で栽培されていた商業品種「マルエイ」の子実体の茎から1973年に分離したものである．#11 から単胞子培養により得られた単核菌糸系統を総称して #11 mono(s) とする．#7 と #5 は1976年に Canada 国 British Columbia 州で採取された野生種で，British Columbia 大学 R. J. Bandoni 博士から分譲された．#7-1 と #7-2 は #7 の分裂子分離培養によって得られた単核系統，#5s5 は #5 から単胞子培養によって得られた単核系統の 1 つである．それぞれの間には，発茸に関する性質においてかなりの差違が認められるので，本実験では検定系統として用いた．複核化したときその発茸能力に貢献する程度について，#7-1 が最も小さく，#7-2 は大きく，#5s5 が最も大きいことが経験的にわかっている．

培養と交配：培養と交配には直径 18 mm 試験管に用意した斜面培地を用いた．培養条件は 25 C，ひとの出入時を除き，暗黒とした．交配には，培地上に供試単核系統 #11 mono(s) と検定系統の菌糸の小塊を互いに少し離して植付けた．両方の菌そうが生長し，接触して，さらに 1 週間以上経過後，#11 mono(s) の左端から (Fig. 1) 菌そうの一部を採り，新しい培地へ植付けた．互いに正逆交配の関係にある複核系統の間では，核の構成が同じでも，菌の形質に差の生ずることがあるので (Kinugawa and Hattori, 1974)，本研究では正逆に交配したもののうち，#11 mono(s) の側から合成複核菌糸を採った．この細胞質は主として #11 に由来すると考えられる．表示法は #11 11s24 (×5s5) などと示し，#11s24 は核受容系統で，かっこ内は供与核を指すものとした．

菌そう生長速度の測定：予備培養した菌そうから菌糸を含む寒天小塊を採り，Fig. 2 に示した変形試験管の水平培地上底から約1/3の位置に植菌した．植付け後 3—4 日目から，10—11日目まで，毎日菌そうの直径を測定した．各系統ともそれぞれ 5 回反復実験を行なった．結果は生長曲線の直線部分の勾配 (tan θ) の平均値で示した (Fig. 3).

発茸能力の測定：複核系統の発茸能力は各検定系統ごとに群内で比較し評価した．その規準は培養の開始から，子実体を収穫するに至るまでの日数およびその収量によって決めた．すなわち，短期間で収穫でき，しかも子実体の収量が大きい系統は優れた発茸能力をもつものとす

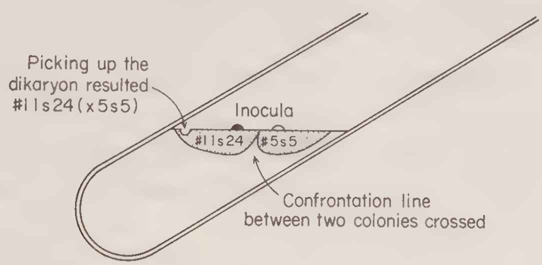


Fig. 1. The agar slant used to cross two compatible monokaryotic strains.

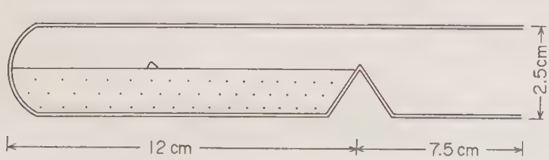


Fig. 2. The horizontal agar medium.

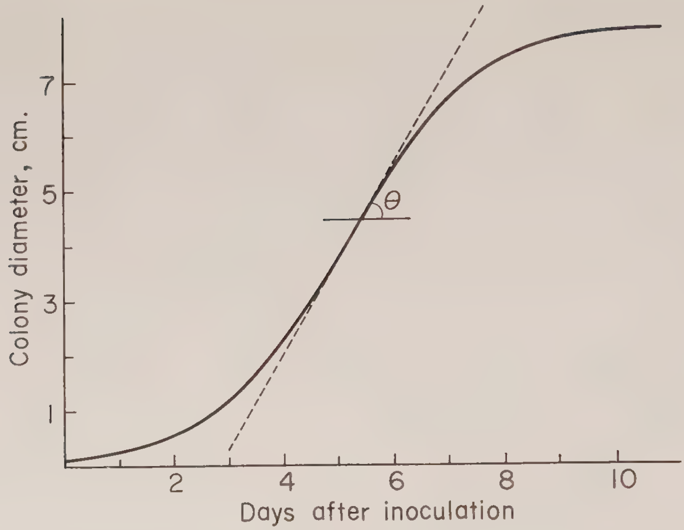


Fig. 3. The expansion of the colony diameter. Expansion rate is determined by tan θ at the point of inflection of the curve.

る (cf. Table 5B). 培養は 100 ml 三角フラスコに準備した 50 ml の液体培地を用い，25 C，毎日数回培養室へのひとの出入によって（5—10分間ずつ）点灯される条件（培地面で 25 lux）下に静置して行った．反復数は各区とも 5 回とした．菌糸体が培地表面全体に拡がったとき，15 C の培養室（光条件は 25 C の室とほぼ同じであるが，照度は 250 lux）へ移し，発茸を誘導し，さらに子実体を発育させた (Aschan-Åberg, 1958; Kinugawa and Furukawa, 1965). 収穫は各フラスコごとに最も生長のよい子実体の傘が開いたときに行ない，培養の開始から収穫までの日数，培地 pH，子実体の長さ，開いた傘の直径，主要な子実体の本数，柄の色などを記録した．収穫した栄養菌糸と子実体は別々に熱風乾燥器（約 40 C）で約 2 週間乾燥させ，乾重を量った．

培地：寒天培地は MYP (Bandoni, 1972) を用いた (Difco malt extract 7 g, Difco soytone 1 g, Difco yeast extract 0.5 g，寒天末（一級）15 g，イオン交換水 1,000 ml, pH 約 6.7)．液体培地は，Ebios 10 g を任意量のイオン交換水で30分間煮沸し，3,600 rpm，10分間遠心分離して，上澄液を取り，グラニュ糖 20 g を加え，イオン交換水で 1,000 ml とした．寒天培地と液体培地はともに 127 C，15分間滅菌した．

オガクズ培地：米ヌカとオガクズ（1：5）を適量の水とともにカップ酒用の瓶（直径 6 cm，高さ 9.2 cm，容量約 160 ml）に詰めた後，培地の中央に底まで穴を開け，紙のふた（中心に小穴のあいたアルミホイル 1 枚をハトロン紙 2 枚の間にはさんだもの）をかぶせ，127 C，約20分間滅菌した．

商業栽培用培地：米ヌカと杉オガクズ（1：5）を適量の水とともに混合し，その 450–480 g を 800 ml p.p. 瓶に詰めた．滅菌は 123 C，1 時間とした．

結　　果

1. ♯11 mono(s) 群および交配による複核系統の菌そう生長速度

Table 2 には ♯11 mono(s) 群およびそれらに 3 種の検定系統を交配して合成した複核系統群の菌そう生長速度の平均値などを示した．♯11 mono(s) の生長速度は，系統によって 0.30—1.15 の間の値を示し，変異係数 [(標準偏差/平均値)×100] は約20%，系統間の変動は比較的大きかった．♯11 mono(s)(×7-2) 群と ♯11 mono(s)(×5s5) 群は似た結果を示した．すなわち，複核系統の菌そう生長速度は少数の例外を除いて，単核系統のそれの 2 倍ほどの値を示した．変異係数はそれぞれ10.00%，13.97%で，単核系統の場合の約1/2 である．これは単核系統が系統間で変動が大きくても，生長のよい複核系統になると互いに類似することを示す．♯11 mono(s)(×7-1) 群は上記の 2 つの群と異り，平均生長速度は ♯11 mono(s) 群とほぼ同じで，変異係数も単核系統の場合より多少高かった．

2. 発茸能力

A. 検定系統 ♯7-2 との交配による複核系統群 [♯11 mono(s)(×7-2)]

Table 3 に ♯11 mono(s) およびそれに ♯7-2 を交配して生じた複核菌糸の生産力測定の結果を示す．都合上，本実験では材料を 2 回に分けて行なった（実験 1 および 2）．対照実験として実験 1 と 2 に用いた系統のいくつかを同時に培養して比較し，実験 3 として表の最下段に示した．Table 3 に示した結果によると，子実体収量は系統間でかなりの差がみられる．Table 5B に示す規準によって複核系統を分類すると発茸能力に関し，a (good)，b (fair)，c (poor) の 3 群に分けられる．♯11s11 と ♯11s24 は単核性発茸能力の高い系統であるが (Table 3)，複核菌糸を合成しても，やはり安定して，比較的高い発茸能力を示した．複核系統の菌そう生長速度と子実体収量（乾重）の間には，相関は認められなかった (Fig. 4)．しかし，発茸能力の高い複核系統を構成している単核系統の tan θ はすべて 0.6 以上の値であったことは興味がある．(子実体収量／総生産量)×100 を子実体生産効率とすると，その値は子実体収量と高い相関を示した (Fig. 5)．子実体収量の多い系統は，概して生産効率の高い系統である．子実体

Table 2. Colony expansion rate of ♯11mono(s)* and synthetic dikaryons (♯11mono(s)(×7-1), ♯11mono(s)(×7-2), and ♯11mono(s)(×5s5))

Experiment No.	Strains	Expansion rate in tan θ** Mean	range	C. V. (%)***
1	♯11mono(s)	0.76	0.30–1.05	21.05
	♯11mono(s)(×7-2)	1.33	1.10–1.54	10.00
	♯7-2	1.10		
2	♯11mono(s)	0.72	0.40–1.05	20.83
	♯11mono(s)(×7-1)	0.80	0.55–1.20	22.50
	♯7-1	0.75		
3	♯11mono(s)	0.72	0.40–1.15	23.61
	♯11mono(s)(×5s5)	1.36	0.70–1.60	13.97
	♯5s5	0.83		

* ♯11mono(s) represents monokaryons originating from basidiospores of a ♯11 fruit body.
** For expansion rate see Fig. 3.
*** C.V.(%)=S.D./Mean×100.

Table 3. Productivity rates of the synthetic dikaryons #11mono(s)(×7–2)* and their component monokaryons

Strain†	Harvest Mean†† (days)	Harvest Range (days)	Dry weight of fruit-bodies(F) (mg)	Dry weight of mycelium (M) (mg)	Final pH of medium	Rank in fruiting ability†††
1.						
#11s1(×7–2)	49.8	33–68	57.2± 7.9**	146.0±12.8**	4.4	b
#11s3(×7–2)	54.8	40–68	70.5± 5.8	164.4±13.7	4.3	a
#11s5(×7–2)	–	28–	55.2± 8.3	231.6±13.0	4.4	b
#11s7(×7–2)	–	46–	61.8±16.8	139.1±14.3	4.2	b
#11s17(×7–2)	36.8	33–44	111.2± 7.6	112.0±12.3	4.4	a
#11s18(×7–2)	–	49–	26.2±15.9	209.5±18.7	4.7	c
#11s19(×7–2)	51.4	38–68	81.2± 8.1	148.7± 8.7	4.2	a
#11s21(×7–2)	51.8	39–68	73.7± 5.4	157.8± 8.7	4.2	a
#11s24(×7–2)	37.8	31–45	90.3± 8.3	137.5±13.8	4.5	a
#11s28(×7–2)	43.6	37–52	87.7±12.0	152.6±13.8	4.4	a
#11s1	–	–	0.	107.4± 2.4	5.2	
#11s3	–	–	0.	165.0±22.1	6.2	
#11s5	–	57–	12.2±10.9	144.8±24.2	5.8	
#11s7	–	–	0.	146.9±10.6	5.3	
#11s17	–	–	0.	158.1±31.9	5.5	
#11s18	–	–	7.8± 3.7	184.1±17.9	6.1	
#11s19	–	–	1.7± 1.7	163.2± 8.4	6.8	
#11s21	–	–	9.5± 3.9	182.6±22.2	5.8	
#11s24	–	–	138.7± 3.8	104.4± 7.3	5.5	
#11s28	–	–	0.	116.6± 3.3	5.1	
#11	55	50–61	70.0± 5.4	118.6± 3.8	4.5	
2.						
#11s2(×7–2)	–	35–	53.2±10.7	205.8±22.8	4.4	b
#11s4(×7–2)	55.5	40–67	48.5± 7.4	121.9± 5.2	4.2	c
#11s8(×7–2)	–	54–	24.9± 5.9	183.3± 7.3	4.3	c
#11s9(×7–2)	–	66–	49.7±12.4	188.8± 8.4	4.2	c
#11s10(×7–2)	39.2	32–44	52.4± 2.3	165.5± 4.7	4.2	b
#11s11(×7–2)	49	38–56	66.3± 2.1	193.9±18.0	4.1	a
#11s12(×7–2)	–	57–	45.3± 7.1	118.0± 4.5	4.3	c
#11s13(×7–2)	51	38–56	71.3± 9.4	171.4±11.3	4.2	a
#11s14(×7–2)	–	66–	29.4± 8.9	217.4±27.4	4.7	c
#11s16(×7–2)	–	57–	42.3±12.9	199.5±13.7	4.4	c
#11s20(×7–2)	44.8	32–61	60.6± 3.1	141.5± 7.6	4.1	b
#11s22(×7–2)	55	43–61	48.0± 7.5	161.2± 5.9	4.3	c
#11s23(×7–2)	49.4	41–63	52.3± 5.2	199.3±18.3	3.8	b
#11s25(×7–2)	–	56–	21.2±11.5	158.1±30.5	4.8	c
#11s27(×7–2)	46.4	37–50	40.5± 3.4	152.7± 5.5	4.1	c
#11s30(×7–2)	–	36–	44.7±10.0	129.7±36.3	4.1	c
#11s31(×7–2)	45	34–61	53.1±13.3	144.5±13.2	4.2	b
#11s2	–	–	0.	205.2± 9.6	6.0	
#11s4	–	–	0.	184.3± 3.5	6.2	
#11s8	–	–	0.	182.1± 2.2	4.8	
#11s9	–	–	0.	167.8± 8.6	6.4	
#11s10	–	–	0.	240.9± 6.2	5.4	
#11s11	–	–	172.9± 7.6	115.4±13.2	5.3	

#11s12	–	–	14.8±11.6	184.0± 5.0	6.0	
#11s13	–	–	0.	196.8± 9.5	5.1	
#11s14	–	–	0.	185.4±28.0	6.1	
#11s16	–	–	0.	110.9± 7.1	6.3	
#11s20	–	–	0.	208.8± 2.8	4.5	
#11s22	–	–	0.	113.1± 3.3	4.8	
#11s23	–	–	0.	210.8± 4.1	5.2	
#11s25	–	–	0.	125.2± 1.2	5.8	
#11s27	–	–	0.	114.9± 3.3	5.3	
#11s30	–	–	0.	110.1± 3.6	5.2	
#11s31	–	–	0.	130.6± 7.5	6.1	
#11	–	–	11.1± 5.2	224.2± 5.6	6.0	
3.***						
#11s11(×7–2)	39.6	34–45	73.2±11.7	193.1±17.0	3.7	a
#11s24(×7–2)	44.6	42–49	87.5± 5.1	181.6± 8.9	3.7	a
#11s27(×7–2)	–	–	0.	116.7± 3.6	5.5	c
#11s22(×7–2)	–	–	0.	122.7± 6.8	6.1	c
#11s8(×7–2)	–	–	1.1± 1.1	168.0±20.8	5.7	c

* See Table 2. ** Mean±S.E. *** Control. † Experimental subjects were separated into three groups and experiments were carried out independently. †† Average of five replicates. ††† Arbitrary ranking.

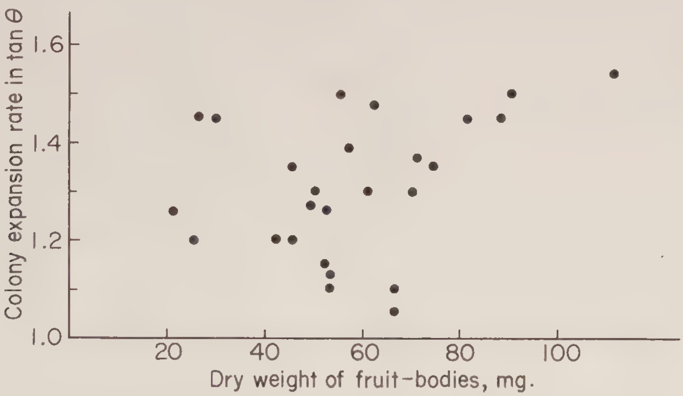


Fig. 4. The relationship between the expansion rate in tan θ of colony diam and fruit-body yield in dry weight of #11mono(s)(×7-2)*. *See Table 2.

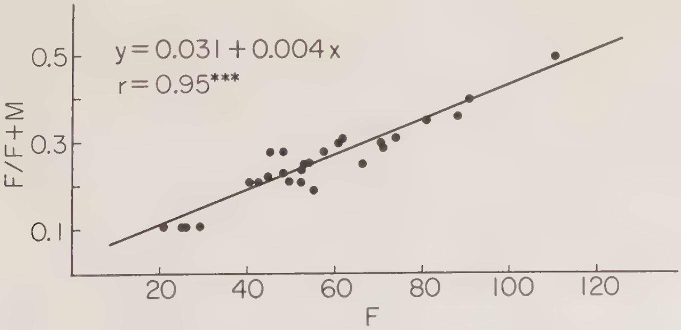


Fig. 5. The relationship between fruiting efficiency rate (F/(F+M)) and fruit-body yield (F) on a dry weight basis, (#11 mono(s)(×7-2)*). M=mycelium yield for dry weight; F+M=total yield. *See Table 2. ***Significant at 0.1% level.

Table 4. Productivity rates of the synthetic dikaryons #11mono(s)(×5s5)* and their component monokaryons

Strain†	Harvest Mean†† (days)	Harvest Range (days)	Dry weight of fruit-bodies(F) (mg)	Dry weight of mycelium (M) (mg)	Final pH of medium	Rank in fruiting ability†††
1.						
#11s2(×5s5)	–	–	0.	152.6± 7.3**	6.7	c
#11s4(×5s5)	–	36–	130.4±18.7**	152.9±23.1	4.3	b
#11s5(×5s5)	–	36–	131.1±11.6	187.6±13.8	4.1	b
#11s11(×5s5)	30	29–31	152.0± 6.8	113.8± 4.3	4.6	a
#11s12(×5s5)	–	31–	49.6±20.2	251.7±29.6	5.1	c
#11s13(×5s5)	38.4	33–42	118.4± 5.3	147.2± 3.6	4.3	b
#11s16(×5s5)	33.4	33–35	149.9±13.6	142.5± 5.1	4.0	a
#11s17(×5s5)	31	29–33	137.0± 5.9	114.3±10.3	4.9	a
#11s18(×5s5)	–	33–	110.0±18.3	192.8±22.7	4.1	b
#11s20(×5s5)	–	35–	91.2±19.6	214.0±22.7	4.5	b
#11s21(×5s5)	–	36–	120.5±17.0	184.9±25.4	4.6	b
#11s22(×5s5)	–	27–	103.5±16.2	179.9±26.2	4.4	b
#11s24(×5s5)***	28.4	27–30	158.0± 7.3	106.9± 4.6	4.3	a
#11s25(×5s5)	37.4	29–44	118.0± 6.7	165.3±14.1	4.3	b
#11s2	–	–	0.	170.1±11.1	6.6	
#11s4	–	–	0.	162.7± 7.2	6.8	
#11s5	–	–	0.	161.5± 6.1	6.8	
#11s11	–	–	159.1±28.2	146.5±10.7	5.9	
#11s12	–	–	0.	157.6± 5.9	5.6	
#11s13	–	–	0.	236.9±19.9	5.6	
#11s16	–	–	0.	138.7± 2.4	5.6	
#11s17	–	–	0.	174.5±17.1	5.4	
#11s18	–	–	0.	142.7±15.9	6.4	
#11s20	–	–	0.	243.9± 4.8	4.2	
#11s21	–	–	0.	133.2± 5.6	5.9	
#11s22	–	–	0.	125.4± 2.0	5.5	
#11s24	–	–	127.3± 8.5	163.3±11.1	6.1	
#11s25	–	–	0.	114.8± 0.8	5.5	
#5s5	–	–	89.5± 5.7	258.1± 6.6	5.8	
2.						
#11s1(×5s5)	–	–	0.	163.4± 8.5	6.2	c
#11s3(×5s5)	–	42–	134.4± 5.8	150.2± 6.2	4.0	b
#11s7(×5s5)	–	31–	62.6±29.1	207.8±30.7	5.1	c
#11s8(×5s5)	–	33–	18.2±18.2	206.9±21.7	5.8	c
#11s9(×5s5)	41	37–45	136.2± 8.3	140.5±13.1	4.3	b
#11s10(×5s5)	–	32–	83.7±23.7	195.8±37.2	4.6	b
#11s14(×5s5)	–	35–	110.2±18.9	183.1±19.6	4.5	b
#11s19(×5s5)	–	33–	102.5±24.9	188.2±28.3	5.3	b
#11s23(×5s5)	–	39–	16.7±15.0	197.7±24.7	5.9	c
#11s24(×5s5)***	35	33–40	175.7± 3.5	117.6± 3.9	4.1	a
#11s27(×5s5)	29.6	28–30	154.6± 8.0	95.4± 4.4	4.6	a
#11s28(×5s5)	–	–	39.5±18.0	274.5±12.0	5.5	c
#11s30(×5s5)	–	46–	58.4±23.6	237.5±22.6	4.8	c
#11s31(×5s5)	–	31–	91.7±23.9	158.3±28.6	4.6	b
#11s1	–	–	0.	139.8± 4.5	6.3	

#11s3	–	–	0.	137.6± 3.8	6.6
#11s7	–	–	0.	170.5± 3.2	5.2
#11s8	–	–	0.	176.4± 7.5	5.4
#11s9	–	–	0.	153.5± 6.4	6.5
#11s10	–	–	0.	152.8± 5.2	6.0
#11s14	–	–	0.	196.0± 8.3	6.2
#11s19	–	–	11.9± 4.1	160.0± 5.1	6.8
#11s23	–	–	0.	247.7± 9.1	5.4
#11s27	–	–	0.	127.5± 2.3	6.3
#11s28	–	–	0.	141.8± 2.7	6.0
#11s30	–	–	0.	118.9± 1.4	6.5
#11s31	–	–	0.	131.6± 2.7	6.2
#5s5	–	–	88.5± 6.4	224.3± 8.9	5.8

* See Table 2. ** Mean±S.E. *** Control. † Experimental subjects were separated into two groups and experiments were carried out independently. †† Average of five replicates. ††† Arbitrary ranking.

Table 5. Classification of #11 monokaryons according to the fruiting ability of synthesized dikaryons

A. Fruiting ability rank.

Rank	#7-1	#7-2	#5s5
a) Good	#11s2, 5, 11, 18, 21, 24	#11s3, 11, 13, 17, 19, 21, 24, 28	#11s11, 16, 17, 24, 27
b) Fair	——	#11s1, 2, 5, 7, 10, 20, 23, 31	#11s3, 4, 5, 9, 10, 13, 14, 18, 19, 20, 21, 22, 25, 31
c) Poor	#11s1, 3, 4, 7, 8, 9, 10, 12, 13, 14, 16, 17, 19, 20, 22, 23, 25, 27, 28, 30, 31	#11s4, 8, 9, 12, 14, 16, 18, 22, 25, 27, 30	#11s1, 2, 7, 8, 12, 23, 28, 30

B. Criteria for monokaryon fruiting potential.

	#7-1		#7-2		#5s5	
	M.h.d.	F*	M.h.d.	F	M.h.d.	F
Rank	(days)	(mg)	(days)	(mg)	(days)	(mg)
a		>0	<52	>65	<35	>137
b				50–65	>35	83.7–137
c	No fruiting		>52	<50	>35	<83.7

* M.h.d., Mean harvest days. F, Dry weight of fruit-bodies. Ranking of monokaryons was determined by comparing mean harvest days (M.h.d.) and dry weight of dikaryon fruit-bodies (F). Refer to Tables 3 and 4 for complete data.

の柄の色は，#11 mono(s)(×7-2) 群全体を通じて，黄褐色に近く，市販品種より濃色であった．#11 mono(s)(×7-2) 群では，培養開始から70日近くを要して収穫されたものが多かった．培地 pH の最終値は複核系統では低く，単核系統または発茸しない複核系統では高い傾向にあった．

B. 検定系統 #7-1 との交配による複核系統群 [#11 mono(s)(×7-1)]

検定系統 #7-1 との交配により生じた複核系統群の発茸能力は非常に低く，70—73日の実験期間中，発茸したのは #11s2(×7-1), #11s5(×7-1), #11s11(×7-1), #11s18(x7-1), #11s21(×7-1) および #11s24(×7-1) の 6 系統だけで，子実体生産量もきわめて少なく，さらに発茸して

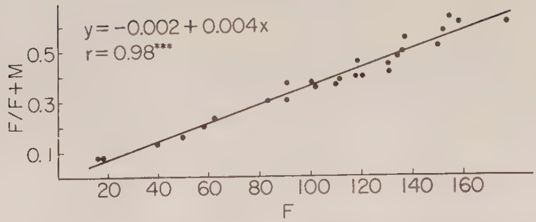


Fig. 6. The relationship between fruiting efficiency rate F/(F+M) and fruit-body yield (F) on a dry weight basis, (♯11mono(s)(×5s5)*). M=mycelium yield for dry weight; F+M=total yield. *See Table 2. ***Significant at 0.1% level.

もほとんどの場合子実体は成熟に至らなかった．しかし，他の系統との比較からこれらは発茸能力としてaに分類した．また，複核系統の多くでは，総生産量も単核系統より少なかった．発茸した複核菌糸を構成する単核系統 ♯11 mono(s) はすべて単核性発茸能力をもつものであり，また菌そう生長速度も $\tan\theta=0.6$ 以上の値を示すものであった．

C. 検定系統 ♯5s5 との交配による複核系統群 [♯ 11 mono(s)(×5s5)]

♯11 mono(s)(×5s5) 群の発茸能力測定の結果を Table 4 に，発茸能力の評価規準を Table 5B に示した．表中，♯11s1(×5s5), ♯11s2(×5s5) など，少数を除き，ほとんどの複核系統は菌糸生長が盛んで，発茸能力も高く，40日以内に収穫されている．これらの複核系統は ♯11 mono(s)(×7-2) の場合と同様，相互の比較から発茸に関して分類し，a, b, c に分けた．ここでも，各複核系統の子実体収量と子実体生産効率とは高い相関をもっていた (Fig. 6).

培養終了後の培地 pH は，検定系統 ♯7-2 の場合と同じく，複核系統で低く，単核系統または発茸しない複核系統で高い傾向にあった．収穫された子実体の柄の色は大体濃茶色であった．

3. 優良な単核系統の選抜

以上，3種の検定系統 ♯7-1, ♯7-2, ♯5s5 と ♯11 mono(s) 群との交配から生じた複核系統は菌糸生長，発茸能力，キノコの形質において大きい変異を示したが，検定系統ごとに分けた群内では供与核はすべて検定系統の核であるから，それぞれの系統に現われる形質は，供試単核系統のもつ遺伝子型を反映しているはずである (cf. Raper and Krongelb, 1958; Esser et al., 1974). 例えば，♯11s17(×7-2) と ♯11s18(×7-2) を比較すると，前者の方がはるかに子実体収量が高い (Table 3). この2系統は交配の片親 ♯7-2 が共通であるから，子実体生産力の差は直接的または間接的に ♯11s17 と ♯11s18 の遺伝的素質の差とみてよいであろう．このようにして，単核系統の発茸潜在能力の優劣を推定し，Table 5 に表示した．Table 5 をみると，多くの単核系統では検定系統が異なれば，発茸能力の大小関係も変って現われていることがわかる．これは恐らく，非相加的な遺伝子効果である雑種強勢の現われ方が違うからであろう．単核系統の中で ♯11s11 と ♯11s24 とは，検定系統が変っても，常に優良な複核菌糸を生じていた．したがって，この2系統は，交配相手が変っても安定して優れた発茸能力を生みだす可能性のある単核系統として，以後の育種過程に用いることができる．一方，♯11s8, ♯11s12 および ♯11s30 は逆に，どの検定系統と交配しても目的とする形質に関し劣った複核菌糸しか生じなかった．

Table 6. Fruit-body production of synthetic dikaryons cultivated on a sawdust rice-bran medium

Strain	Average no. of fruit-bodies per culture*	Length of fruit-bodies (cm)	Pileus diam (cm)	Average fresh weight per culture (g)*	Average ratio of colored portion of stalk	Days to harvest
#11s24(×5s5)	72.6	5.5–11.5	0.8×1.0–2.5×2.5	23.1	0.77	40
#11s24(×7-2)	32.3	0.8–10.5	0.5×0.5–2.4×2.5	9.5	0.73	41
#11s11(×7-2)	36.2	1.8–12.5	0.3×0.3–2.8×2.8	12.3	0.73	41
#11s17(×7-2)	20.7	1.5–11.0	0.6×0.6–3.3×4.0	7.3	0.57	44
#11s13(×7-2)	2.6	5.0– 8.0	1.4×1.5–2.1×2.5	1.5	0.75	52

* Average of ten replicates.

Table 7. Productivity of #11s24(×5s5) cultivated by the Yamanouchi Enokitake Cooperative in Osaka*

Stock	Pileus diam (mm)	Fresh weight (g)**	Length of stipe (cm)
#11s24(×5s5)	6×6–8×11	79.4±2.0***	11.9±0.2
#11	2×2–4× 4	44.5±1.8	5.0±0.2
Yamanocuhi commercial strain	4×5–7× 8	64.5±2.4	9.9±0.2

* Calculated from a total of thirty-two culture bottles (800 ml).
** Per bottle.
*** Mean ±S.E.

4. 実用栽培による優良系統の発茸能力の測定

前節の結果から選出した #11s11 と #11s24 を交配親とする複核系統および比較のための系統をオガクズ培地で栽培し，発茸能力を調べた．結果を Table 6 に示す．子実体の収穫は傘が大体開いたときに行ない，傘の直径，子実体の長さ，生じた子実体本数，新鮮重と柄の着色部の長さについて調査した．各系統は発茸の時期が違うために，収穫日も違っている．調べた系統のうち，#11s13(×7-2) はこの培地での発茸能力が低かった．他の 3 系統は中間で，#11s24(×5s5) は最も優れていた．#11s24(×5s5) は菌そうの生長が最も速く，子実体の新鮮重，本数ともに，供試した系統中一番大きい値を示した．

次に，#11s24(×5s5) を大阪府下，山内エノキタケ生産組合で試験栽培した結果を Table 7 に示した．#11 は本実験の素材となった複核菌糸 (Table 1) で，#11s24(×5s5) は #11 よりも，また現在の実用品種よりも，生産能力が高いことがわかる．この結果は，育種のこの時点における 1 つの到達点である．なお，この栽培は，栽培過程で生育の揃った栽培ビンを選ぶ作業を行なっていないため，収量は通常の栽培における見かけの収量より低くなっている．

考　　察

Brodie (1948) は *Cyathus stercoreus* の複核菌糸体の菌糸生長速度は単核菌糸体の約 2 倍であると報告した．本実験でも，#7-2 および #5s5 と合成した複核系統群において，これと同じ現象を認めた (Table 2)．衣川，小原 (1968)，小原 (1968) は 1 つの子実体の担子胞子に由来する単核菌糸系統群を相互に交配して複核菌糸系統群を合成した．それらを比較して，菌糸生長のよい複核系統は子実体形成においても優れていることを認めた．本研究の結果では，発茸

能力と菌糸生長の間には有意な相関はなかったものの，発茸能力の優れた系統（単核系統およびその合成複核系統）は菌糸生長も概ねよいものの中にあることが認められた．例えば，発茸能力が高い複核系統を集め，その受容核親である ♯11 mono(s) の菌そう生長速度を調べるとすべて $\tan\theta$ が0.6以上であった．すなわち，栄養生長の悪い単核系統は，全体として，生産能力の低いものであるから，育種上，当初から期待できないようである．空中菌糸量や菌糸密度が発茸能力とどのような関係にあるかについては，はっきりした結論を得ることができなかった．

Esser et al. (1974) によれば，*Agrocybe aegerita* では複核菌糸の発茸能力と単核菌糸の単核性発茸の間に統計上有意な相関があるという．♯7-1 と交配して合成した複核系統のうち，発茸に至った系統の片親，すなわち ♯11s2, ♯11s5, ♯11s11, ♯11s18, ♯11s21, ♯11s24 はすべて単核性発茸能力をもち，とくに ♯11s11 と ♯11s24 において顕著であった．以上のように，エノキタケでは単核性発茸能力をもつ単核菌糸が常に優れた発茸能力の複核菌糸を生ずるとは限らないが，それらは一般によい育種素材として期待できそうである．

複核系統の発茸能力におけるもう1つの要因は菌糸生産量 (M) と子実体生産量 (F) の関係である．子実体生産量と菌糸生産量を加えて総生産量とすると，合成複核系統群 ♯11 mono(s)(×7-2) と ♯11 mono(s)(×5s5) では，それぞれ 200–250 mg と 250–320 mg との比較的狭い範囲内にある．しかし，子実体生産量と菌糸生産量はそれぞれの群内で大きい変動を示し，ほぼ子実体生産が多いと菌糸量は少なくなる傾向がある．すなわち，両者の値は総生産をどのように分割するかで決まり，分割比が系統によって大きく異なる．Figs. 5 および 6 は，総生産量のうち子実体生産量が占める割合（子実体生産効率：F/(F＋M)）と子実体生産量 (F) の関係を示しており，1つの点は1つの系統の平均値である．図は F/(F＋M) と F の間の強い相関関係を示す．すなわち，F/(F＋M) の値が大きければ，子実体の生産量が多くなる．以上のことから，総生産量が多く，そのうえ子実体生産への配分の大きい（子実体生産効率の高い）複核系統を発見することと，そのような効果を生ずる単核系統の組合せを見いだすことが育種上大切であることが分る．

予備実験の結果から，単核系統 ♯7-1 は発茸に必要な因子をあまりもっていない，♯7-2 はもっているが少ない，♯5s5 は比較的たくさんもっている，とそれぞれ考えて選定したものである．なるべく少数で，発茸と子実体形質に関し特性の違う検定系統を用いることによって，供試単核系統の中から常に安定して有用な特性を示す系統を効率よく捜し出すことができれば，育種の作業量を軽減することになる．

♯11 mono(s)(×7-2), ♯11 mono(s)(×7-1) および ♯11 mono(s)(×5s5) 3群の複核系統群の発茸能力は，群内でも，群間でも，かなり大きい変動を示した．結果において述べた通り，群内の変動は主として ♯11 mono(s) 群の遺伝的形質の差，群間の差は主として検定系統間の遺伝的差によるものとみてよいであろう．供試単核菌糸や検定系統の恐らく量的形質を支配する遺伝子による素質の上へ，遺伝子の相互作用による雑種強勢や，逆に相互作用による発茸能力の弱化 (Negative heterosis; Jones, 1952) などが重なって複核系統群の発茸に関する特性をつくりあげているものであろう．Table 5A において数種の単核系統が選出されたが，これらは複核系統の発茸能力に対する単核系統の寄与の大小を検定系統を用いてかなり効率よく推定し得ることを示し，Raper and Krongelb (1958) の結果とも一致した．

以後の育種作業としては，検定交配によって選抜した単胞子株同志を交配して，優良な複核菌糸を生ずる組合せを見いだせば育種の第1サイクルを終ることになる．

参考のため，本実験で作成した複核菌株の一部を使用してオガクズ培養（実験室内および工場）におけるキノコ生産量を比較した．これは選抜の効果が実用栽培においてどのように現われるか調べることにもなる．液体培養でも特に成績のよかった #11s24（×5s5）は，オガクズ培地および工場での商業栽培においても，元の複核菌株 #11 に比べ，子実体の生産量が高く，比較的短期間で収穫できた（Tables 6, 7）．オガクズ培養では発茸能力の差が液体培養の場合よりも明らかに現われるようである．この系統をすぐ実用品種として用いることはできない．発茸能力に関してはすでにかなりの水準に到達しているが，子実体の着色度が強いため，商品価値が低いのである．

摘　　要

エノキタケを用い，効率的で，便利な食用キノコの育種法を見いだすことを目的として研究を行なった．まず，カナダの野生エノキタケから分離した子実体形成能力に関し特性の違う3種の単胞子株を検定子株として選び，これを日本の栽培品種より分離した27単胞子株と交配した．次に，できた複核菌株の菌そう生長および発茸能力に関する特性を比較し，各単胞子株の発茸に関する潜在能力を推定した．この方法を用いると，育種素材として有望である単胞子株を比較的簡単に，また有効に選出することが可能であった．

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アフラトキシン産生性 *Aspergillus flavus* と非産生菌との米混合培養における非産生菌のアフラトキシン産生抑制効果

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Haruo TSUBOUCHI, Katsuhiko YAMAMOTO, Kazuo HISADA, Yoshio SAKABE, and Kazuyoshi TSUCHIHIRA*: Inhibitory effects of non-aflatoxigenic fungi on aflatoxin production in rice cultures by *Aspergillus flavus*

Summary

The effects of two non-aflatoxigenic fungi, *Aspergillus niger* and *A. flavus* (a non-aflatoxigenic strain), on aflatoxin production by *A. flavus* were studied. Conidia mixtures containing different conidia ratios of the two strains (e.g. the aflatoxigenic strain of *A. flavus* vs. *A. niger*: the aflatoxigenic *A. flavus* vs. the non-aflatoxigenic *A. flavus*) were inoculated into rice media. For 3×10^4 conidia of the aflatoxigenic *A. flavus* per 50 g of rice, conidia of each strain of non-aflatoxigenic fungus were added to provide the following three ratios: 10 : 1, 1 : 1 and 1 : 10 in the inoculum mixtures. After inoculation, the rice was incubated at 28 C for 18 days and the amounts of aflatoxins produced were analyzed at 3, 6, 9, 12, 15 and 18 days. Rice inoculated with only the aflatoxigenic *A. flavus* was also incubated and analyzed as the control.

Zero or low levels of aflatoxins were detected when the rice was simultaneously inoculated with conidia of the combination of aflatoxigenic *A. flavus* and *A. niger* or that of the aflatoxigenic and the non-aflatoxigenic strains of *A. flavus*. In the culture with *A. niger*, reduction of aflatoxins B_1 and G_1 was more rapid and effective than that in culture with the non-aflatoxigenic strain of *A. flavus*. After 6 days, the production of aflatoxins B_1 and G_1 in rice inoculated with conidia of equal amounts of the aflatoxigenic *A. flavus* and *A. niger* (1 : 1) decreased conspicuously as shown in Table 1. For the control, the reductive rates of aflatoxins ranged from 95.9 to 96.3% (B_1) and from 94.2 to 95.2% (G_1) respectively. Moreover, no aflatoxins were detected in rice inoculated with a 1 : 10 ratio of the aflatoxigenic *A. flavus* to *A. niger* after incubation at 28 C for 6 days or longer.

食糧，食料品および飼料などのマイコフローラの調査において，検出された真菌がその基質上で該当するマイコトキシンを産生するかどうか検討するとき，その基質に共存する他のマイコトキシン非産生菌の影響についても考慮する必要がある．

すなわち，1）非産生菌による産生菌の発育抑制に伴うマイコトキシン産生への影響，2）産生マイコトキシンに対する非産生菌の分解作用である．特にアフラトキシン（以下 AF と略す）に関しては，AF 汚染が重視されるピーナツ，トウモロコシ，香辛料などのマイコフローラを調査する際，*Aspergillus flavus* Link（AF 産生株および非産生株とも）と *A. niger* van Tieghem がよく共存する事実に遭遇する．両者とも発育に必要な最低水分活性がそれぞれ a_w 0.84 および 0.86 であることから（宇田川，鶴田，1975），同一の基質において同時にこれ

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らの菌が発育し，しかも *A. niger* などの非産生菌が AF 産生株の発育を抑制し，その結果として AF の産生に影響を及ぼすことも予測される．*Aspergillus flavus* の AF 産生株に対する各種の真菌による抑制効果は Ashworth (1965) によるピーナツの仁を用いての観察があり，*A. niger* が AF 産生を抑制することを認めているが，詳しくは報告されていない．さらに，Boller and Schroeder (1973, 1974) の報告によれば玄米培地に *A. parasiticus* Speare と *Eurotium chevalieri* Mangin および，*A. parasiticus* と *A. candidus* Link ex Fr. を混合着生させた場合には，玄米における AF 産生量が *A. parasiticus* 単独培養時に比べて明らかに低いことを報告している．また，鶴田ら (1977) の研究ではトウモロコシを基質として，AF 産生性 *A. flavus* と *A. ochraceus* Wilhelm, *A. restrictus* G. Smith および *E. chevalieri* を共存させて培養した場合，*A. flavus* 単独培養時より異菌種共存時にはその AF 産生量が低減することを報告している．また，Mann and Rehm (1977) および坪内ら (1980) は真菌による AF B_1 の分解試験を液体培地を用いて実施し，特に *A. niger* によって AF が分解されることを明らかにした．

これらのことより，今回 *A. flavus* の AF 産生株および AF 非産生株，AF 非産生菌としての *A. niger* を選び，これらの菌が混合着生した場合の AF 産生について白米培地を用いて比較検討を行った．

実験材料および方法

1. 培地 精白米の一定量をとり約2時間，水に浸したのち，篩を用いて水切りし，300 ml 容三角フラスコに 50 g ずつ採取した．綿栓後，121 C, 15分間高圧滅菌し，培地とした．滅菌後の白米の水分は「厚生省，105° 加熱による乾燥法 (1973)」によって測定したところ，31.1% であった．

2. 供試菌株 AF B_1, B_2, G_1, G_2 を産生する *A. flavus* として，中国産トウガラシより分離した S-42-1-1株〔以後 *A. flavus* (AF +) と略記する〕，AF 非産生菌として同じく中国産トウガラシより分離した *A. niger* S-42-4-3株およびパキスタン産トウガラシより分離した *A. flavus* S-43-4-3株〔以後 *A. flavus* (AF −) と略記する〕をそれぞれ使用した．なお，最後の *A. flavus* (AF −) については上記白米培地を用いて予め試験を行い，AF を産生しないことを確認した．

供試菌株は PDA 斜面培地で 25 C，1週間培養したのち，これに滅菌した 0.05% "Tween 80" 水溶液を加え，よく振とうし，150メッシュの金網を用いて菌糸塊などを沪別・除去して，分生胞子原液とした．原液の一部をとり，血球板で分生胞子数を計測し，*A. flavus* (AF +) の胞子浮遊液は 6×10^4/ml に，*A. niger* および *A. flavus* (AF −) の胞子浮遊液はともに 6×10^3/ml, 6×10^4/ml, 6×10^5/ml の3段階の濃度になるよう滅菌 0.05% "Tween 80" 水溶液で希釈，調製した．以上の各胞子浮遊液は 5 C に保管し，使用に当って室温に戻して用いた．

3. 培養

1) *Aspergillus flavus* (AF +) と *A. niger* との混合培養

A. flavus (AF +) の胞子浮遊液 (濃度 6×10^4/ml) と *A. niger* の胞子浮遊液 (濃度 6×10^5/ml) の各 0.5 ml を上記 300 ml 容三角フラスコ中の白米 50 g に加え，混合比 1：10の接種とした．同様に *A. flavus* (AF +) の胞子浮遊液 (6×10^4/ml) と *A. niger* の胞子浮遊液 (6×10^4/ml) の各 0.5 ml を白米 50 g に加え混合比 1：1 の接種，*A. flavus* (AF+) の胞子浮遊液 (6×10^4/ml) と *A. niger* の胞子浮遊液 (6×10^3/ml) の各 0.5 ml を白米 50 g に加え混合比10：1 の接種とし

た．上記のいずれの場合も *A. flavus* (AF ＋) の接種量は，50 g の基質に対して 3×10^4 個の分生胞子数となっている．以上，各3段階の混合比の接種を行った白米培地のそれぞれを 28C, 18日間培養し，経時的に AF 量を測定した．通常，各測定には2個の培養物を使用した．

2) *Aspergillus flavus* (AF ＋) と *A. flavus* (AF －) との混合培養

上記 1) と全く同様の操作によって，両株の接種に際しての混合比 1：10, 1：1, 10：1 の3種類の接種を行った白米培地のそれぞれを 28 C, 18日間培養し，経時的に AF 量を測定した．

3) 対照区

Aspergillus flavus (AF ＋) の胞子浮遊液 (6×10^4/ml) と滅菌0.05% "Tween 80" 水溶液の各 0.5 ml を接種した白米培地を対照とした．

4. **再現性実験** これらの実験の再現性が見られるかを調べるために，上記と全く同じ条件の培養を時期をかえて反復した．

5. **AF の分析** 培養3日目ごとに培養物につき「厚生省，環食第128号通達 (1971)」の方法に準じて AF の定量を行った．すなわち，培養物にメタノール：1%塩化ナトリウム水溶液 (55:45, v/v) を 200 ml あて加え，そのまま一夜放置したのち，ウルトラ・トラックスで十分にかくはん，細切し，次にこれを沪紙（東洋沪紙製，No. 5A）で沪過した．沪液 20 ml を等量のクロロホルムで3回抽出後，クロロホルム層を分取，無水硫酸ナトリウムで脱水，減圧下で濃縮乾固した．抽出物にベンゼン：アセトニトリル (98:2, v/v) 5 ml を加えて溶解後，同一溶媒で希釈し薄層クロマトグラフィー (TLC) により比色定量した．TLC プレートは Biosil A（Bio-Rad 社製）に5%の割合にギプスを加えたもの（20×20 cm, 厚さ 0.25 mm）および DC-Fertigplatten Kieselgel 60 (Merck 社製，20×20 cm, 厚さ 0.25 mm) をそれぞれ，110 C, 1時間活性化して使用，クロロホルム：アセトン（9：1, v/v）で展開した．なお，この場合の定量限界は AF B_1 および G_1 とも 0.05 ppm であった．測定結果は2個の培養物の平均値で示した．

6. **培養物の pH 測定** *Aspergillus flavus* (AF ＋) と *A. niger* の混合培養物，*A. flavus* (AF ＋) と *A. flavus* (AF －) 1：1 の混合培養物および *A. flavus* (AF ＋) 単独培養物について，6日目と15日目の pH を測定した．その方法は培養物に 100 ml のイオン交換水を加え，ウルトラ・トラックスで十分にかくはん，細切したのち，No. 5A 沪紙で沪過し，沪液を pH メーターで測定した．

7. **接種菌株の生育状況** *Aspergillus flavus* (AF ＋) と *A. niger* の混合培養物については，それぞれの菌の生育状態を知るため，以下の方法により比較を試みた．その方法は，6日目と12日目の培養物に 200 ml の滅菌 "Tween 80" 水溶液を加え十分に振とうしたのち，上層の主として分生胞子浮遊液の部分を150メッシュの金網で出来るだけ菌糸塊や共存する異物を沪別・除去した後，滅菌 "Tween 80" 水溶液で約 10^6—10^7 倍に希釈し，PDA 平板（9 cm ペトリ皿）培地に 0.5 ml ずつ塗まつし，25 C で *A. flavus* および *A. niger* のコロニーが出現し識別できるまで培養し，両菌種のコロニー数の比を求めた．

結　　果

I. AF 産生量の消長

第1回の実験結果を Fig. 1 および Fig. 2 に示した．

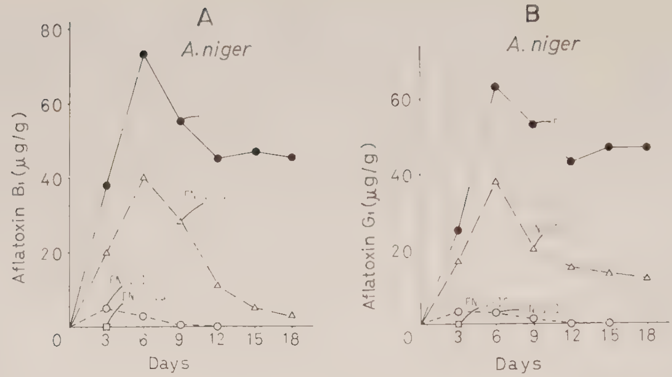


Fig. 1. The effect of the simultaneous inoculation of aflatoxigenic *A. flavus* and *A. niger* on the production of aflatoxins B_1 (1-A) and G_1 (1-B) in rice cultures incubated at 28 C. Ratio of *A. flavus* conidia to *A. niger* conidia in the inoculum: FN 10-1—10: 1 (3×10^4/50 g rice of *A. flavus*: 3×10^3/50 g rice of *A. niger*), FN 1-1—1 : 1 (3×10^4/50 g rice each of *A. flavus* and *A. niger*), FN 1-10—1: 10 (3×10^4/50 g rice of *A. flavus*: 3×10^5/50 g rice of *A. niger*), F—Control (3×10^4/50 g rice of *A. flavus* only).

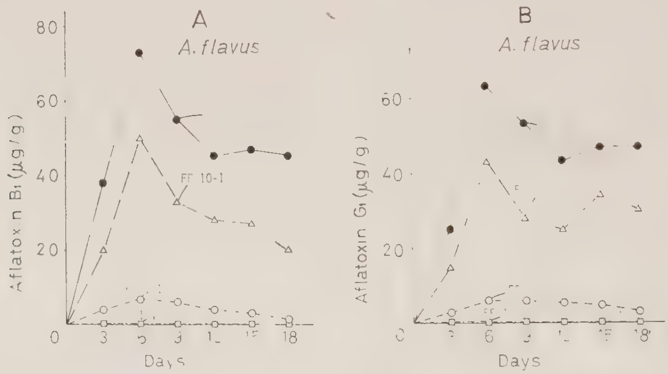


Fig. 2. The effect of the simultaneous inoculation of aflatoxigenic and non-aflatoxigenic strains of *A. flavus* on the production of aflatoxins B_1 (2-A) and G_1 (2-B) in rice cultures incubated at 28 C. Ratio of aflatoxigenic *A. flavus* conidia to non-aflatoxigenic *A. flavus* conidia in the inoculum: FF 10-1—10 : 1, FF 1-1—1 : 1, FF 1-10—1 : 10, F—Control (All amounts of conidia are similar to those used in Fig. 1).

1. *Aspergillus flavus* (AF +) と *A. niger* との混合培養

1) AF B_1 について

Fig. 1-A のように混合比10：1で接種した場合には3日目に産生量は20 μg/g を，6日目には最高値40 μg/g を示した．そして，9日目以後は減少傾向を示した．また，混合比1：1で接種した場合には，3日目に最高値5 μg/g を，6日目から減少傾向を示し，12日目以後は検出されなかった．さらに，混合比1：10で接種した場合には，3日目に痕跡 (<0.05 μg/g) を示したのみで，以後は検出されなかった．

2) AF G_1 について

Fig. 1-B のように混合比10：1で接種した場合，3日目に17 μg/g，6日目に最高値38 μg/g を示し，9日目以後は減少傾向がみられた．混合比1：1で接種した場合には，3日目に最高値

3.5 μg/g を，6日目以後は減少を始め，15日目以後は検出されなかった．また，混合比1：10で接種した場合，3日目に痕跡（<0.05 μg/g）を検出したのみで，以後は検出されなかった．

2. *Aspergillus flavus* (AF ＋) と *A. flavus* (AF −) との混合培養

1) AF B_1 について

Fig. 2-A のように混合比10：1で接種した場合，3日目に 20 μg/g，6日目には最高値 50 μg/g を示し，9日目以後対照区に平行するようにじょじょに減少傾向がみられた．また，混合比1：1で接種した場合は，3日目に 4 μg/g，6日目に最高値 7 μg/g を示し，9日目以後はじょじょに減少をみたが，18日目でも 1.5 μg/g を示した．混合比1：10で接種した場合には，AF B_1 量はわずかながら（痕跡—0.19 μg/g）18日間を通じて検出された．

2) AF G_1 について

Fig. 2-B のように混合比10：1の接種区では，対照区の推移と平行するように AF G_1 の産生量の増減が観察された．混合比1：1接種区では，AF B_1 の場合と同じように6日目に最高値 6 μg/g を示し，以後じょじょに減少をみたが，18日目でも 3 μg/g を示した．混合比1：10の接種区では，微量（痕跡—0.19 μg/g）ながら18日間を通じて AF G_1 が検出された．

以上の AF の産生量の消長は第2回の実験においても，ほぼ同様な結果となった．また，本実験では AF B_2 と G_2 についても測定し，ほぼ AF B_1 および G_1 と同じような結果が得られたが，AF B_2, G_2 ともその産生量が少なかったため記述を省略した．

II. *Aspergillus flavus* (AF ＋) 単独接種区と AF 非産生菌との混合接種区との AF 産生量の比較

対照区の AF 産生量と *A. flavus* (AF ＋) と *A. niger* および *A. flavus* (AF ＋) と *A. flavus*

Table 1. The reductive effects of the simultaneous inoculation of aflatoxigenic *A. flavus* and *A. niger* or non-aflatoxigenic *A. flavus* on the production of aflatoxin B_1 in rice cultures incubated at 28 C

Inoculum composition (aflatoxigenic: non-aflatoxigenic)		Reduction percentages of B_1 after 3 to 18 days of incubation* 3	6	9	12	15	18
A. flavus: *A. niger*	10:1	47.4	45.2	54.5	75.6	89.5	93.3
		14.3	44.4	72.2	68.0	92.5	93.3
	1:1	86.8	95.9	99.2	100	100	100
		85.7	96.3	99.2	100	100	100
	1:10	100	100	100	100	100	100
		99.8	100	100	100	100	100
A. flavus: *A. flavus*	10:1	47.4	31.5	40.0	37.8	38.9	62.2
		33.3	17.9	18.3	13.3	30.8	23.1
	1:1	89.5	90.4	89.1	91.1	93.7	96.7
		77.0	67.9	75.0	53.3	84.6	61.5
	1:10	99.6	99.8	99.8	99.8	100	100
		97.5	97.1	98.8	99.1	97.8	97.7

* Data are based on duplicate cultures in each cultural condition.
Reduction percentages calculated as follows: 1–Aflatoxin B_1 amounts produced by the simultaneous inoculation and growth of aflatoxigenic *A. flavus* and *A. niger* or non-aflatoxigenic *A. flavus*/Aflatoxin B_1 amounts produced by growth of aflatoxigenic *A. flavus* only ×100.

Table 2. The reductive effects of the simultaneous inoculation of aflatoxigenic *A. flavus* and *A. niger* or non-aflatoxigenic *A. flavus* on the production of aflatoxin G_1 in rice cultures incubated at 28 C

Inoculum composition (aflatoxigenic: non-aflatoxigenic)		Reduction percentages of G_1 after 3 to 18 days of incubation* 3	6	9	12	15	18
A. flavus: *A. niger*	10:1	32.0	39.7	62.3	65.0	71.6	74.7
		16.7	33.3	40.0	60.0	70.0	65.7
	1:1	86.0	95.2	97.5	99.6	98.9	100
		83.3	94.2	97.0	98.4	99.7	100
	1:10	100	100	100	100	100	100
		99.8	100	100	100	100	100
A. flavus: *A. flavus*	10:1	40.0	31.7	47.2	41.9	28.4	36.8
		28.6	40.0	38.1	21.7	28.6	14.3
	1:1	88.0	90.5	88.7	87.2	90.0	93.7
		62.9	84.0	81.2	56.5	64.3	57.1
	1:10	99.2	99.8	99.8	99.8	99.9	100
		94.9	98.4	98.5	98.9	96.4	96.4

* See foot-note in Table 1. Reduction percentages calculated on Aflatoxin G_1 instead of B_1 in Table 1.

(AF −) との混合培養時の AF B_1 および G_1 産生量を比較するため，対照区のそれぞれの産生量に対する減少率に換算し，Table 1, Table 2 に示した．

1．AF B_1 について

A. flavus (AF ＋) と非産生菌の混合比10：1についてみると，*A. niger* を混合培養した場合には，3日目に47.4％および14.3％を示し，その後ほぼ経時的にその減少率は上昇し，15日目以後には第1回，第2回とも約90％以上となった．一方，*A. flavus* (AF −) を混合培養した場合には，3日目から18日目までその減少率は第1回の実験における18日目を除き，ほとんど40％以下での変動に終った．混合比1：1については，*A. niger* を混合培養した場合，3日目に86.8％および85.7％を示し，その後減少率は上昇し，12日目以後には100％を示した．一方，*A. flavus* (AF −) との混合培養では，18日間を通じて53.3％以上96.7％までの間を変動した．混合比1：10については，*A. niger* を混合培養した場合には，3日目以後18日目までほとんどすべて減少率100％を示した．一方，*A. flavus* (AF −) との混合培養では3日目よりすべて97％以上の減少率を示したが，18日間を通してほとんどが100％にはならなかった．

2．AF G_1 について

AF B_1 とほぼ同じ結果を示した．しかし，*A. niger* との混合比10：1および1：1の接種区では，その減少率の経時的な上昇度が，AF B_1 のときに比し低い傾向を示した．

Ⅲ．培養物の pH と接種菌株の生育状況

Aspergillus flavus (AF ＋) と *A. niger* との10：1，1：1，1：10各混合培養物，*A. flavus* (AF ＋) と *A. flavus* (AF −) との1：1混合培養物および対照区としての *A. flavus* (AF ＋) 単独培養物のそれぞれについて，6日目と15日目の pH を Table 3 に示した．表より，*A. niger* の接種量が多い場合には6日目，15日目とも有機酸の産生に伴う酸性化が著しかった．

Aspergillus flavus (AF ＋) と *A. niger* との混合培養物の6日目と12日目のそれぞれの分生胞

Table 3. Changes in pH in rice cultures as affected by the ratio of the conidia of aflatoxigenic *A. flavus* to the conidia of *A. niger* or non-aflatoxigenic *A. flavus* in the inoculum

Inoculum composition (aflatoxigenic: non-aflatoxigenic)		pH after 6 or 15 days of incubation at 28 C	
		6	15
A. flavus: *A. niger*	10:1	4.7	4.0
	1:1	3.2	3.2
	1:10	3.1	3.0
A. flavus: *A. flavus*	1:1	6.0	5.2
Control (aflatoxigenic *A. flavus* only)		5.5	6.0

Table 4. Comparison of growth ratio in rice cultures between aflatoxigenic *A. flavus* and *A. niger* after simultaneous inoculation and incubation

Inoculum composition (*A. flavus*: *A. niger*)	Growth ratio (*A. flavus*: *A. niger*) after 6 or 12 days of incubation at 28 C	
	6	12
10:1	30:1	50:1
1:1	1:3	3:1
1:10	1:20	1:60

子を主とする測定値の比を Table 4 に示した．両菌ともに接種後 3 日目には分生子頭は小型であり，分生胞子の形成が観察されたが，まだ未熟であった．そして，6 日目には分生子頭も大型となり，分生胞子の成熟も進み，以後の観察では変化がみられないようであった．表より明らかなように，混合比10：1，1：10いずれも接種量の多い方の菌が時間経過とともに始めの比率よりも 5—6 倍優勢となる傾向がみられた．

考　　察

結果に示したように，*A. flavus* (AF ＋) を *A. niger* あるいは *A. flavus* (AF —) と混合培養した場合，明らかに AF 産生量が減少した．また，AF 非産生菌の接種量を多くするほど，AF 産生量の減少が強められ，とくに混合比が 1：1 および 1：10において著しい AF 産生量の減少が確かめられた．例えば，*A. flavus* (AF ＋) と *A. niger* 混合培養では，対照区の AF 産生量が最高に達する培養 6 日目における AF B_1 の減少率は混合比 1：1 の接種区において平均96.1%，混合比 1：10の接種区において100%を示した．また，*A. flavus* (AF ＋) と *A. flavus* (AF —) 混合培養では，1：10接種区での培養 6 日目における減少率は AF B_1, G_1 とも平均98.5% および99.1%を示した．

一方，AF 産生の時間的経過に関しては，*A. flavus* (AF ＋) と *A. niger* との混合培養と，*A. flavus* (AF＋) と *A. flavus* (AF —) との混合培養における AF B_1 および G_1 の産生量の消長を比較すると，前者ではいずれも AF B_1 および G_1 の減少率が時間経過とともに上昇する傾向を示したのに対し，後者では AF B_1, G_1 のいずれについても，ほぼ最初の培養 3 日目の減

少率に近い数値を18日間を通じて示した.

このように *A. niger* と *A. flavus* (AF —) との間に，AF 減少率の差がみられるのはどのような理由に基づくのであろうか．*A. niger* および *A. flavus* (AF —) は液体培地で AF B_1 の産生に対する影響が明らかにされているが，両者には違いがあり，*A. niger* の場合は AF B_1 の分解であり (Mann and Rehm, 1977; 坪内ら，1980)，*A. flavus* (AF —) の場合は主に AF B_1 の菌体への吸着であることが報告されている (Masimango et al., 1979)．したがって，これらの菌を用いて固体培養を行ったとき，単に 2 種類の菌の混合培養時における菌株間の生育競合による AF 産生量の低下だけが AF 測定値の減少につながるとはいえないものと考えられる．すなわち，上記のように，*A. niger* との混合培養において 6 日あるいは 9 日目以後の AF B_1, G_1 の減少には *A. niger* の AF 分解性が関与している可能性もあるという考えが成立する．著者ら（坪内ら，1980）の研究によれば，AF B_1 を 1 ppm 添加した Czapek-Dox 液体培地で *A. niger* を培養した場合，*A. niger* の産生する有機酸により AF B_1 は AF B_{2a} となり分解する現象と，培地に炭酸カルシウムを加えて pH を調整して培養した場合には，AF B_{2a} に変換しないで AF B_1 が減少する現象のあることがみられた．今回の実験において，*A. flavus* (AF＋) と *A. niger* の 1：10混合培養の場合，15日目に pH は3.0を示したが，AF B_{2a} は検出されなかった．しかし，AF B_{2a} は一度生成されても培地中のたんぱく質と結合して抽出されない (Ayodeji et al., 1977) のか，あるいは全く生成されないかは検討する必要がある．いずれにしても，*A. niger* の生育が AF の分解に何らかの関与をしていることが推定される．

液体培地を用いた *A. flavus* (AF —) との混合培養における AF 産生量の減少は上記のようにその一部が菌体吸着によるものと考えられるが，本実験では菌体中の AF も同時に抽出しているから，*A. flavus* (AF —) の菌体吸着による影響は測定値にそれほど現われていないものと考えられる．したがって，*A. flavus* (AF ＋) と *A. flavus* (AF —) の混合接種では，両者間の生育における競合によって，産生株の生育が抑制された分だけ AF 産生量が減少したものと推定される．Masimango et al. (1979) はモロコシ粉を基質として，*A. flavus* AF 産生株と同非産生株を 1：1 の混合比で接種，培養したが，菌株間による抑制力の差が若干あるとはいえ，その実験結果と今回の結果とは大体の点において一致が認められた．

Doyle and Marth (1978) は *A. parasiticus* AF 産生株を単独で培養したとき，培養後 5 — 18日の菌体に AF B_1 自己分解が認められ，9 日目のものが最高の能力があるとし，とくに AF 産生能の高い株ほど自己分解能も高いと報告している．今回の実験でも，対照区の 9 日目以後 AF B_1, G_1 の測定値が減少している．この *A. flavus* (AF ＋) の AF 自己分解能について言及すると，*A. niger* との混合培養において *A niger* が AF 産生株の AF 自己分解能に何らかの促進的効果を及ぼした結果，競合の影響に加えて AF 測定値がさらに減少するということも考えられるが，この点については今後検討する必要がある．

以上のように白米培地上で *A. flavus* (AF ＋) と *A. niger* または *A. flavus* (AF —) などの非産生菌との混合培養により AF 産生量の減少をみたことは，実際に野外での真菌汚染でも同様な現象が起こりうるものと想像される．しかし，今回の実験では定量分析上の都合から白米を基質とした点で，実際の AF 汚染が重視されるピーナツ，トウモロコシ，香辛料など異なる基質によっても認められるものであるかどうかについては，今後の検討が必要である．また，*A. flavus* と *A. niger* が野外において同時に上記の基質上に侵入するかどうかの点についても疑

問が残る．例えば，ピーナツにおける *A. flavus* の侵入時期はきわめて早いことが報告されている．一方，分離された菌株のAF産生能が非常に高いにもかかわらず，その菌を分離した試料からは全くAFが検出されないか，検出されても非常に微量である例がしばしばみられる．基質の成分がAF産生に対し抑制的である場合や，基質上でAFが産生される条件が十分に満されない場合もあるが，AFなどマイコトキシン産生菌に他の菌が共存した場合，その産生量が減少したり，産生されたマイコトキシンが分解されたりする可能性も十分に考えられる．

摘　　要

白米培地に *Aspergillus flavus* (AF＋) の分生胞子 $3\times10^4/50$ g および *A. niger* または *A. flavus* (AF－) の分生胞子を混合比10：1，1：1，1：10の3段階になるようにそれぞれ混合接種し，28 C，18日間培養，AF B_1 と G_1 の産生量を *A. flavus* (AF ＋) 単独培養（対照区）の場合と経時的に比較し，つぎの知見を得た．

1) AF B_1 および G_1 ともに産生量は，*A. flavus* (AF ＋) の単独培養時の産生量に比べて減少した．とくに *A. niger* との混合培養では，*A. flavus* (AF －) との混合培養に比し，より著しい減少が認められた．

2) *A. flavus* (AF ＋) と *A. niger* を混合比1：1および1：10の割合で接種，培養した場合，対照のAF B_1 および G_1 量が最高値に達する培養6日目での産生量減少率は1：1接種区でAF B_1 が平均96.1%，AF G_1 が平均94.7%を示し，1：10接種区ではAF B_1, G_1 とも100%を示した．また，混合比1：1の接種では，AF B_1 が12日目以後，AF G_1 が18日目に検出されなくなり（減少率100%），また混合比1：10の接種では，AF B_1, G_1 とも6日目以後検出されなかった．

3) *A. flavus* (AF ＋) と *A. niger* との共存がAF産生に及ぼす影響について考察した．

終わりに，本研究に際して終始御指導を賜わった国立衛生試験所真菌室長　宇田川俊一博士に深謝いたします．

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書　評

Carmichael, J. W., W. B., Kendrick, I. L. Conners, and L. Sigler: Genera of Hyphomycetes. 386 p. The University of Alberta Press, Edmonton, Canada. 1980. US $ 21.00.

古くは Lindau, Clements and Shear, 最近では Barnett, Barron などによって出されてきた不完全菌，特に Hyphomycetes の図集に関する成書がついにここに至ったというべき書である．本書は Kendrick と Carmichael によって The Fungi IVA (1973) にすでに示された図を引続き追加検討した結果，一冊の書としてまとめられたものである．

緒言でのべられている本書刊行の目的は，1) Hyphomycetes の属までの検索，2) Hyphomycetes として報告されてきたすべての属名のもうら，3) 各属における重要参考文献の引用，の 3 つである．この目的にそうため，次の項目によって本書が構成されている．1) Hyphomycetes の属の列記(A, B, C 順)，2) 本書に採用した全属の代表的な 1　2 種の特徴を示す図，3) 分類のための検索表．緒言につづき属，種の意義と考察，命名法，分生子の形質表示などが述べられているが，重要な点は，本書では従来採用されてきた菌類の命名出発点に関し，Hyphomycetes では Hughes (1958) の意見を採用し，Persoon の Synopsis Methodica Fungorum (1801) によった点である．これは従来出発点として用いられてきた Fries (1821) と混同を起しそうであるが，そのため1821年以前に用いられた属名はリストが示されている．詳細は本書で述べられているが，さほどの混乱はないものと思われる．何より役立つのは，A, B, C 順に示された属のリストで，学名，発表年，原文献，図の有無（発表当時），タイプ種，synonyms とその文献などを主体とし，さらに分生子の諸形質の記号化が付されている点である．また，本書において採用した属はゴチック体で区別されている．分生子の形質は，Saccardo 方式によるものと分生子形成様式によるものとに分け，first & second key-word として示されている．1801年以後のものとして，2032属が載せられている．

次に図であるが，すべての引用した属にわたっているわけではないが，採用した属についてはかなり詳しい図が載せられている．もちろん，著者らのオリジナル，原著の転用など混ざっているが，属の特徴を知るうえには便利であろう．図の次に検索表があるが，著者ら独特の検索表で分生子形成様式を正確に把握した場合には大いに役立つ．

本書の目玉となる図は合計853におよび，原著からの書き直しが主体であるが，線は統一されており，並べ方にも苦心がうかがえる．ただし，必ずしもタイプ種の図ではなく，また各属の構成種の形態には巾広いものもあり，ある程度の予備知識が属の決定には要求されよう．本書によってのみ属を決定するというのではなく，座右の書として利用すべきであろう．Hyphomycetes の分類に関心のある者は，ぜひ備えておくべき書である．

(Keisuke Tubaki　椿　啓介)

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Aspergillus fumigatus とその近縁菌における痙れん性カビ毒: fumitremorgin A, B の生産性

堀 江 義 一・山 崎 幹 夫*

Yoshikazu Horie and Mikio Yamazaki*: Productivity of tremorgenic mycotoxins, fumitremorgins A and B in *Aspergillus fumigatus* and allied species

Summary

In order to establish their ability to produce the tremorgenic mycotoxins, fumitremorgins A and B (FTA, FTB), 104 strains of *Aspergillus fumigatus*, previously isolated from substrates including soil, food, herbal drugs and diseased human tissues, were examined using rice cultures at 25 C for 21 days. Moldy rice was extracted with ethyl acetate, and fumitremorgins in the extracts were confirmed by thin-layer chromatography (TLC). The TLC plates were coated with Silica Gel G (Merck) and benzene-acetone (4 : 1, v/v) was used as a solvent. The amounts of fumitremorgins were determined by measuring the intensity of their blue fluorescence after spraying 20% $AlCl_3$ in ethyl alcohol (80 C, 20 min) under ultraviolet light.

Only three strains of *A. fumigatus* out of the total 104 were positive for FTA production, but 98 strains in the same screening test were found to produce FTB. Most strains produced very high levels of FTB (4.8 to 3,052.0 mg/kg), but for the few strains producing FTA, the production levels were very low (0.9 to 11.6 mg/kg).

To determine whether fumitremorgins are also produced by other species of the' *A. fumigatus*' group (Raper and Fennell, 1965), one strain each of *A. brevipes*, *Neosartorya aurata*, *N. stramenia* and *Neosartorya* sp., fifteen strains of *N. fischeri* var. *fischeri*, three strains of *N. fischeri* var. *glabra* and two strains of *N. quadricincta* were tested using the same cultural and analytical procedures.

Fumitremorgins were found not only in the cultures of two strains of *N. fischeri* var. *fischeri* and the *Neosartorya* sp. strain, but FTA production by these positive strains was noticeably high (172.9 and 195.4 mg/kg). Thus, fumitremorgin production of *Neosartorya* spp. has been demonstrated for the first time.

Fumitremorgin A および B (FTA, FTB) は実験動物の中枢神経系に作用し，強い振せん，痙れんを起こすマイコトキシンで，1971年に著者らが宮城県古川市周辺の農家より集めた自家製の味噌から分離された *Aspergillus fumigatus* Fres. の代謝産物として発見された（山崎ら，1971a; Yamazaki et al., 1971b)．類似のマイコトキシンとしては *Penicillium verruculosum* Peyronel の代謝産物 verruculogen, acetoxy verruculogen, *A. fumigatus* の代謝産物 TR-2, fumitremorgin C (FTC), epoxide FTC が報告されている．FTA, FTB の化学構造は，その後の研究で Fig. 1 のように決定されている (Yamazaki et al., 1974; 1975a, b)．すなわち，

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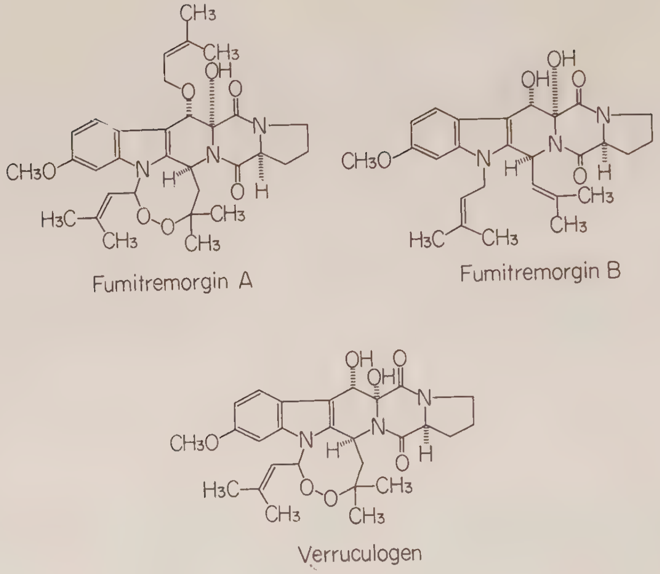


Fig. 1. Structures of fumitremorgins A, B and verruculogen.

verruculogen, TR-2, FTC, epoxide FTC とともに，いずれも 6-methoxytryptophan と proline からなる dioxopiperazine を基本骨格とする一連の化合物である．また，FTA, FTB ともに，さらに数種の *Aspergillus*, *Penicillium* からの生産が報告されている．

A. fumigatus は穀類をはじめとし，各種の食品，生薬，土壌など自然界に広く分布することが知られている (Semeniuk, 1954; Gilman, 1957; 宇田川，鶴田，1975; 堀江ら，1977, 1979; 山崎ら，1980)．しかし，FTA, FTB などの生産菌の自然界における分布と生産性についての研究は，これまでほとんどみられない．そこで，著者らは FTA, FTB の主生産菌種 *A. fumigatus* とその近縁菌について生産性の検索を行った．

試料および実験方法

供試菌株 *A. fumigatus* は土壌，食品，生薬，人体などの各基質より分離された104菌株を用いた．土壌分離株は秋田県大曲市，同仙北郡角館町，同中仙町，宮城県古川市，同玉造郡岩出山町，同鳴子町，千葉県八千代市，同佐倉市，鹿児島県姶良郡霧島町などの水田，畑地，森林，草地土壌から分離された41株を供試した．食品分離株はマレーシア（クアラセランゴール州）の農家保有米由来の20株，同塩蔵魚より分離された1株の合計21株を供試した．生薬分離株はアカメガシワ，アキノキリンソウ，エンメイソウ，オウレン，カゴソウ，カンゾウ，キキョウ，キョウニン，シツリシ，シテイ，ジュウヤク，セネガ，チコッピ，チョウトウコウ，トウキ，ニワトコヨウ，ヒトツバハギ，ブクリョウ，ヘンチク，ヤクモソウ，リュウタンなど21種類の生薬より分離された35株を供試した．人体由来株としては当研究所保存の7株を用いた．

また，Raper, Fennell (1965) の *A. fumigatus* 菌群に含まれる *Aspergillus brevipes* Smith, *Neosartorya aurata* (Warcup) Malloch & Cain, *N. fischeri* (Wehmer) Malloch & Cain var. *fischeri* Malloch & Cain, *N. fischeri* var. *glabra* (Fennell & Raper) Malloch & Cain, *N. quadricincta* (Yuill.) Malloch & Cain, *N. stramenia* (Novak & Raper) Malloch & Cain, *Neosartorya* sp. など24株も供試した．これらの供試菌株は内外保存機関から分譲をうけたもの，土壌，生薬（ケンパク，セネガ，ゾクダン，ニンジン）より分離されたものである．

培養と FTA, FTB の検出　500 ml 容ルーフラスコに十分吸水させた精白米 200 g を入れ，121 C, 20 min 滅菌後，供試菌株を接種し，25 C, 21日間培養した．なお，ルーフラスコは各供試菌株あたり 2 本使用した．培養終了後，酢酸エチルで抽出，減圧濃縮した抽出エキスをメタノールに溶解し，石油エーテルを用いて脱脂後，メタノール層を濃縮し，得られた試料を薄層クロマトグラフ (TLC) による分析に供した．TLC プレートはシリカゲルG（メルク社，20×20 cm)，展開溶媒としてベンゼン：アセトン (4：1, v/v) を用いた．FTA, FTB の検出は20%塩化アルミニウム：エタノール溶液 (w/v) を噴霧し，80 C, 10 min 加熱後，UV ランプ下で青色螢光スポットとその Rf 値を測定し，FTA, FTB 生産の有無を判定した．

FTA, FTB の定量　上記の結果によって FTA, FTB のいずれかを生産することを確認した菌株のうち，FTA, FTB をともに生産する 5 株と，FTB のみを生産する20株，合計25株を選び以下の試験を行なった．

これらの菌株の酢酸エチル抽出エキスを FTA, FTB の標品 (FTA は 0.2 μg, 0.5 μg, 1.2 μg, FTB は 0.06 μg, 0.3 μg, 0.5 μg をそれぞれ使用）とともに TLC プレート上にスポットし，上記と同様の方法で TLC を行った．試料のスポット量は，検量線の直線性を示す範囲として，FTA については 0.1–1.2 μg, FTB については 0.03–0.5 μg となるように希釈調整した．測定には螢光装置つき 2 波長クロマトスキャナー（島津製作所 CS-910 型）を使用し，FTA は励起波長 380 nm，螢光波長 460 nm，FTB は励起波長 355 nm，螢光波長 460 nm で，それぞれ 450 nm のフィルターを用いて定量した．本法による検出限界量は FTA において TLC スポットあたり 0.1 μg, FTB において 0.03 μg であった．

結　　果

1. *A. fumigatus* における FTA, FTB の生産性

Table 1 に示すように，土壌由来の供試41株中の FTA 生産菌は宮城県土壌より分離された

Table 1. Production of fumitremorgins by *Aspergillus fumigatus* strains isolated from various substrates

Isolation source	Number of strains tested	Number of fumitremorgins producing strains	
		A	B
Soil	41	1	40
Foods	21	2	21
Herbal drugs	35	0	31
Diseased human tissues	7	0	6
Total	104	3	98

Table 2. Production of fumitremorgins by *Aspergillus brevipes* and *Neosartorya* spp. strains

Species	Number of strains tested	Number of fumitremorgins producing strains	
		A	B
Aspergillus brevipes	1	0	0
Neosartorya aurata	1	0	0
Neosartorya fischeri var. *fischeri*	15	1	2
Neosartorya fischeri var. *glabra*	3	0	0
Neosartorya quadricincta	2	0	0
Neosartorya stramenia	1	0	0
Neosartorya sp.	1	1	1

Table 3. Yields of fumitremorgins by rice cultures of 25 representative strains of *Aspergillus fumigatus* and *Neosartorya* spp.

Species	Strain no.	Source	Fumitremorgins (mg/kg)	
			A	B
A. fumigatus	77-MY-45-1	Soil	ND*	2,368.4
A. fumigatus	77-MY-50-2	Soil	ND	2,400.3
A. fumigatus	77-MY-51-1	Soil	11.6	447.8
A. fumigatus	77-MY-59-1	Soil	ND	2,212.7
A. fumigatus	77-MY-66-1	Soil	ND	3,052.0
A. fumigatus	78-TO-76-1	Soil	ND	1,414.2
A. fumigatus	78-TO-86-2	Soil	ND	233.4
A. fumigatus	78-TO-89-2	Soil	ND	1,418.9
A. fumigatus	78-TO-114-1	Soil	ND	1,359.5
A. fumigatus	79-SH-300-3	Herbal drug	ND	306.7
A. fumigatus	79-SH-335-6	Herbal drug	ND	4.8
A. fumigatus	79-SH-377-2	Herbal drug	ND	227.3
A. fumigatus	79-SH-427-1	Herbal drug	ND	546.1
A. fumigatus	79-SH-429-1	Herbal drug	ND	254.0
A. fumigatus	79-SH-437-8	Herbal drug	ND	284.5
A. fumigatus	79-SH-454-10	Herbal drug	ND	405.1
A. fumigatus	79-SH-460-9	Herbal drug	ND	377.2
A. fumigatus	79-SH-510-18	Herbal drug	ND	83.8
A. fumigatus	79-SH-512-6	Herbal drug	ND	139.7
A. fumigatus	79-SH-520-10	Herbal drug	ND	66.0
A. fumigatus	C-18	Rice	ND	25.0
A. fumigatus	C-26	Rice	Trace	775.9
A. fumigatus	C-45	Rice	0.9	14.0
N. fischeri var. *fischeri*	79-SH-413-1	Herbal drug	172.9	177.4
Neosartorya sp.	79-SH-335-11	Herbal drug	195.4	35.9

* ND. Not detected.

株のみであったが，FTB は40株に生産性が認められた．FTB 生産菌の地域別ひん度（生産株数／供試株数）は，それぞれ秋田県 11/11，宮城県 15/15，千葉県 9/9，鹿児島県 5/6 であった．食品由来株では FTA 生産菌は米から得た 2 株のみであったのに対して，FTB は供試した21株すべてに生産性が認められた．生薬由来株には FTA 生産菌は発見されず，一方 FTB の生産は供試35株中31株に認められた．人体由来株も同様に FTA 生産菌は認められず，FTB の生産菌は供試 7 株中 6 株に認められた．以上をまとめると，FTA 生産は全供試菌株の2.9%に，FTB は94.2%に認められた．

近縁菌における FTA, FTB の生産性

A. fumigatus 近縁菌における FTA, FTB の生産性検索の結果を Table 2 に示した．FTA 生産菌はニンジンから分離された *N. fischeri* var. *fischeri* とケンバクから分離された *Neosartorya* sp. の 2 株に認められ，一方 FTB 生産菌はこれら FTA 生産菌に加えて *N. fischeri* var. *fischeri* の 1 株，合計 3 株に認められた．

A. fumigatus とその近縁菌の FTA, FTB 生産量

FTA, FTB 生産性 *A. fumigatus* と *Neosartorya* spp. について，精白米培地上における生産量を調べ Table 3 に示す結果を得た．*A. fumigatus* における FTA の生産量は精白米 1 kg あたり 0.9–11.6 mg であった．また，*N. fischeri* var. *fischeri* は 172.9 mg/kg, *Neosartorya* sp. は 195.4 mg/kg の生産量を示した．一方，FTB の生産量は 4.8–3,052.0 mg/kg の範囲を示し，菌株によって大きな差異が認められた．特に，土壌由来の *A. fumigatus* は，食品・生薬由来の株と比較して高い生産量を示す株が多数認められた．また，*N. fischeri* var. *fischeri* の生産量は 177.4 mg/kg, *Neosartorya* sp. の生産量は 35.9 mg/kg であった．

考　　察

Fumitremorgin，およびその関連マイコトキシンによると思われる家畜中毒の発生がオーストラリア，ニュージーランド，イギリスなどから報告され，特に羊の ryegrass stagger 症候群中毒が注目されている．これらのうちで，FTA による中毒の原因菌としては *Penicillium janthinellum* Biourge の関与が報告され（Lanigan et al., 1979; Giesecke et al., 1979），また FTB による中毒の場合は *P. piscarium* Westling が原因菌とされている（Gallagher and Latch, 1980; Gallagher et al., 1977）．さらに，verruculogen による中毒ではこの両種のほか，*P. estinogenum* Komatsu & Abe も原因菌と報告されている（Day and Mantle, 1980; Day et al., 1980）．なお，Patterson et al.（1979）は ryegrass stagger 症候群中毒の一要因として報告された toxin X が FTB と同一物質の可能性があると述べている．Toxin X の生産菌としては，*P. nigricans* (Bain.) Thom, *P. piceum* Raper & Fennell, *P. raistrickii* Smith, *Penicillium* sp. および *N. fischeri* が知られている（Patterson et al., 1979; Shreeve et al., 1979）．このほか，ryegrass stagger 症候群中毒のマイコトキシンとして *P. cyclopium* Westling など数種の *Penicillium* が生産する penitrem A も知られている．

以上のように *A. fumigatus* による家畜の ryegrass stagger 症候群中毒の報告はこれまでみられなかったようであるが，しかし *A. fumigatus* が世界各地に広く分布すること（Raper and Fennell, 1965），また本研究の検索結果から供試菌株の94.2%に FTB の生産性が認められたこと，特に土壌由来の多数の株に Table 3 に示すように高生産性が認められたことから，今

後 *A. fumigatus* に汚染された飼料，牧草などが原因となって ryegrass stagger 症候群様の中毒事故が発生する可能性は十分に予測されるものと考えてよかろう．なお，今回の供試菌株中には verruculogen 生産株は認められなかった．

近縁菌 *Neosartorya* spp. での FTB の生産性菌株の検出率は13%であり，*A. fumigatus* と比較するときわめて低い結果を示した．しかし，FTA 生産量においては Table 3 にみられるようにその高生産性に特徴が認められる．また，*A. fumigatus* とほぼ同様の条件で生育を示すことから，やはりマイコトキシンを少なからず生産し，中毒の原因となり得るものと考えられ，注目された．

FTA, FTB などの定量については，Day et al. (1980) の *P. estinogenum* による verruculogen の簡易定量法，Gorst-allman and Steyn (1979) の FTB 定量法が報告されている．しかし，培養による FTA, FTB の生産に関して定量まで行なった報告はこれまでみられず本報告が最初である．

本研究の一部を担当された千葉大学生物活性研究所活性天然物化学部鈴木重紀博士，大口井純氏，および試料採集にあたり協力をえた宮城県衛生部の関係者に感謝いたします．

摘　　要

土壌，食品，生薬，人体などから得た *A. fumigatus* 104株について fumitremorgin A, B (FTA, FTB) の生産性を検索した．その結果，3株に FTA，98株に FTB の生産性が認められた．同様に関連菌24株について生産性を検索した結果，*N. fischeri* var. *fischeri* 1株と *Neosartorya* sp. 1株に FTA 生産性，さらに他の *N. fischeri* var. *fischeri* 1株からも FTB 生産性を認めた．これらの生産菌について TLC および2波長クロマトスキャナーを用いて FTA, FTB の生産量を定量した結果，精白米培地 1 kg あたりの FTA 生産量は 0.9–195.4 mg, FTB は 4.8–3,052.0 mg の範囲を示した．

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新 刊 紹 介

平井篤造他編：日本植物病理学史. 261頁. 日本植物病理学会，東京. 1980. 2,800円.

本書は1975年に日本植物病理学会創立60周年記念として発刊が提案された．その後，方針，内容などの検討を重ね，専門分野別に執筆を分担し，このたび上梓されたものである．

同学会ではすでに会報31巻記念号で，植物病害研究の展望と問題点，研究技術史，学会史などが記述されている．しかし今回発行された病理学史は，すべての分野を網羅し，各項目の内容も充実していることなどから，今までにない本格的なものといえる．もうひとつの特徴は，単に事項の年代別整理ではなく，読物としてもすぐれていることであろう．

第１編「緒論」　植物病理学が明治時代に導入されて以来，多くの先達の努力と協力により，わが国に根ざした学問として，地道に発展してきたさまが，簡潔に，しかも相互に関連づけながら記述されている．

第２編「植物病理学研究史」　ウイルス・マイコプラズマ，細菌，菌類，線虫，生理病，感染，疫学，育種，薬剤，検疫の各項目にわたって，現在までの研究史と今後に残された課題が述べられている．

第３編「植物防除研究史」　イネ，ムギ・雑穀，いも・まめ類，野菜類，果樹，観賞植物，特用作物，牧草，樹木の項目に分け，それぞれの重大な病害について，研究史と問題点が述べられている．

第４編「その他の活動，事業史」　日本植物病理学会の活動（組織，大会，学会賞など），試験研究組織の発展，国際協力の発展について述べられている．

「人物史」　ト蔵梅之丞博士はじめ22名の人物史で，故人とかかわりの深かった執筆者が，尊敬と思慕をこめて記述している．

「年表」　いくつかの項目別に詳しい年表がつけられている．

以上，目次に沿って内容にふれた．

最近のように試験研究の内容が，高度に専門化，細分化されてくると，ともすると自分の仕事が全体の中で，どの位置にあり，どういう役割を果たそうとしているのかを見失いがちである．また，専門分野がどのような発展をとげて現在にあるのかを忘れがちとなる．今一度，自分の仕事を整理しなおす意味でも，特に若い研究者の座右に置きたい書である．植物病理学専攻以外の，菌学を専攻されている方々も是非御一読ください．

(Hiromichi Horie　　堀江　博道)

Trans. mycol. Soc. Japan (日菌報) 22: 121-126, 1981

SHORT COMMUNICATION

Scanning electron microscopic observations of a fungal invasion of corn kernels

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鶴田　理・郷原　茂・斉藤　道彦*：トウモロコシ粒へのカビ侵入に関する走査顕微鏡観察

Summary

Invasions of healthy harvested corn kernels by hyphae of *Aspergillus candidus*, *A. flavus*, *A. restrictus*, *Eurotium chevalieri* and *Penicillium aurantiogriseum* were observed under a scanning electron microscope. All the fungi tested began colony development on the surface at the center of the germ and on the tip cap. In the case of invasions through the germ center, only a few hyphae penetrated the pericarp covering the germ despite the formation of abundant mycelia on the surface, and spread from the invasion site into the germ. The invasion of *A. restrictus* hyphae from the tip cap to the germ was prevented by the kernel hilar layer. However, the hyphae of the four other fungi invaded the kernels and spread from the tip cap through the germ to the endosperm, mostly via the space between the pericarp and the germ.

It has been established that fungal invasion of kernels during storage is a major cause of damage to corn that is to be used as foods and feeds. This damage by fungi during storage occurs first in the germ of the kernel (Christensen and Kaufmann, 1969, 1974). Qasem and Christensen (1960) first demonstrated that injuries to the pericarp covering the germ facilitated the entry of fungi into the corn kernel, and suggested that if the pericarp remained unbroken storage fungi might enter the germ, probably through the point of attachment, that is the tip cap.

Since then, however, little work has been done on this invasion process. In this study, the modes of invasion of corn kernels by five common storage fungi were compared using a scanning electron microscope.

Materials and Methods

1. **Fungi**. Strains of *Aspergillus candidus* Link, *A. flavus* Link, *A. restrictus* Smith, *Eurotium*

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chevalieri Mangin and *Penicillium aurantiogriseum* Dierckx, were isolated from imported corn kernels as described previously (Tsuruta et al., 1972, 1974), and used for the following observations.

2. **Inoculation and incubation.** Healthy dent corn kernels were sterilized in CO_2 with 30% ethylene oxide for 8 h at 25–30 C. Sterilized kernels were inoculated with spores of fungi by the following method: Ten kernels of corn in 5 cm diam Petri dish were mixed thoroughly with ten kernels of rough rice on which abundant spores of each fungus had been produced after a two-week incubation period. The rice kernels were then removed from the Petri dish. Inoculated corn was incubated at 26 C, and about 98% R.H. in the containers.

3. **Observations**. Seven to 10 days after inoculation, molded samples were fixed in 2% glutaraldehyde for 18 h and postfixed in 1% osmium tetroxide for 2 h. After fixing, the kernels were dehydrated successively in 70, 80, 90, 95 and 100% ethanol. The kernels were dried with a Hitachi HCP-1 critical-point dryer, coated with a film of gold with an Eiko IB-3 ion coater, and then examined under a Hitachi-Akashi MSM-4 scanning electron microscope.

Results and Discussion

The fungal invasion of corn kernels generally began at the tip cap and in the center of the germ (Figs. 1, 2). In the case of an invasion by *A. candidus* of the germ, even though abundant mycelia grew on the surface, only a few hyphae penetrated the pericarp and then spread into the germ (Fig. 3). The method of invasion was identical in the four other fungi species tested. The pericarp covering the center of the germ was thinner than that covering the other parts (Figs. 4, 5), but this could not explain sufficiently why it was here that the invasion began.

Corn kernels connect with the cob at their tip caps which consist of a sponge-like structure without a cuticle, and provide the major path of moisture into the kernels. The structure of the tip cap permits the ready growth of the hyphae of many common storage fungi if there is a favorable environment. Although the growth at the tip cap

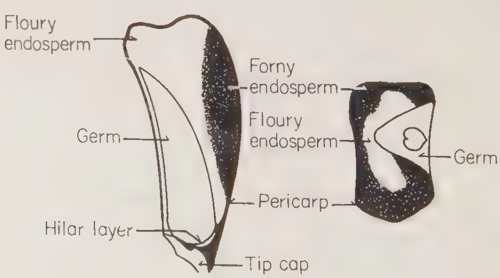
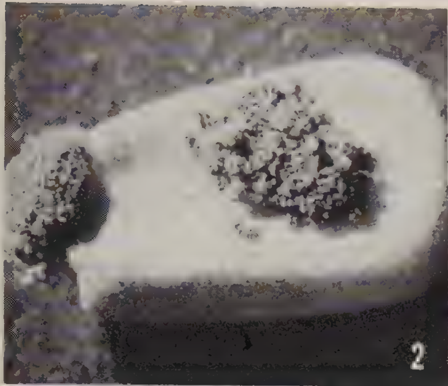
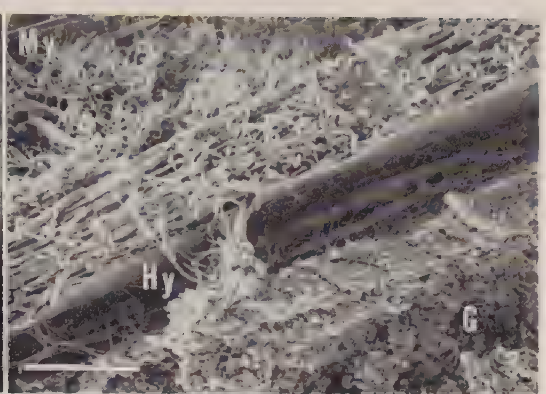
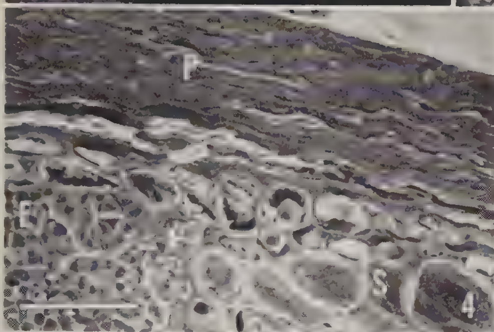


Fig. 1. Structure of mature corn kernel (Dent corn).







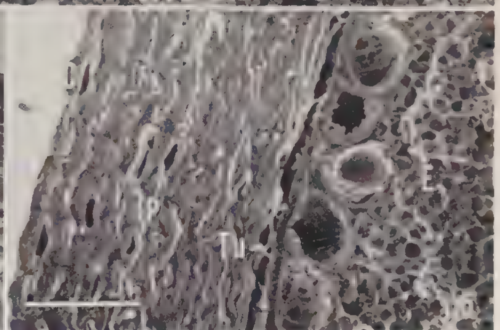




Fig. 2. Fungal colonization at the sites of invasion of corn kernel.

Fig. 3. Invasion of a corn kernel with *A. candidus* through the pericarp (P) covering the germ, showing a few hyphae (Hy) which have penetrated despite the formation of abundant mycelia (My) on the surface. After penetration, the fungus spread into the germ (G).

Fig. 4. Pericarp (P) covering the center of the germ. E: endosperm and S: scutellum.

Fig. 5. Pericarp (P) covering the horny endosperm (E). C: cuticle and Tu: tubular cells.

Fig. 6. Invasion of a corn kernel with *A. restrictus* through the tip cap (T), showing hyphae (Hy) prevented by the hilar layer (H) from extending. S: scutellum.

Scales: 3 = 100 μm; 4, 5 = 50 μm; and 6 = 200 μm.



Figs. 7-10

of the five different fungi tested did not differ, the degree of invasion of the kernels from the infection site varied markedly.

As Fig. 6 shows the hyphal invasion of the germ by *A. restrictus* from the tip cap is prevented by the hilar layer which may act as a barrier. However, further experiments with different isolates of *A. restrictus* are needed to confirm this. In the case of *E. chevalieri*, hyphae invaded from the tip cap through the space between the pericarp and the germ to the end of kernel, and cleistothecia formed in this space (Figs. 7, 8). The hyphae of *A. flavus* and *A. candidus* invaded the kernel from the tip cap to the endosperm via the germ. Particularly vigorous growth of *A. flavus* was observed (Fig. 9). Although the hyphae of *P. aurantiogriseum* also spread from the tip cap into the germ (Fig. 10), whether the hyphae had developed through the germ itself or in the space between the pericarp and the germ could not be confirmed.

The present observations have shown that the mode of invasion of corn kernels by fungi varied greatly among the five strains tested. Further investigations are necessary to clarify the behavior of many other contaminative fungi which cause the spoilage of corn arriving at the market.

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摘　　要

収穫されたトウモロコシ整粒への *Aspergillus candidus*, *A. flavus*, *A. restrictus*, *Eurotium chevalieri* および *Penicillium aurantiogriseum* の菌糸の侵入過程を走査顕微鏡により観察した.

供試菌はすべて，胚芽中央部の表面と尖帽部でコロニーを形成し始めた.

Fig. 7. Invasion of a corn kernel with *E. chevalieri* through the tip cap (T), showing mycelia (My) spreading vigorously into the space between the pericarp (P) and the germ (G). H: hilar layer.

Fig. 8. Cleistothecia (Cl) of *E. chevalieri* which have formed beneath the pericarp (P). G: germ.

Fig. 9. Mycelia (My) of *A. flavus* which have formed near the coleoptile (Co) in the germ after the invasion of mycelia through the tip cap. S: scutellum.

Fig. 10. Mycelia (My) of *P. aurantiogriseum* on the germ surface (G) beneath the pericarp (P).
Scales: 7=500 μm; 8, 9=200 μm; and 10=500 μm.

胚芽中央部からの侵入の場合には，表面に多量の菌糸体が形成されているにもかかわらず，果皮を貫通する菌糸はきわめて少数であり，菌糸はその侵入部から胚芽中へ広がった．

A. restrictus の尖帽部から胚芽への侵入はトウモロコシ粒のハイラー層により阻止された．これに対して，尖帽部からトウモロコシ粒に侵入した他の 4 供試菌の菌糸は，主に果皮と胚芽の間隙を通って胚芽から胚乳へと広がった．

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資　料

日本国内にも発生したミツバチの chalk brood disease

古谷　航平*・高鳥　浩介**・園部　修***・馬渕　貞三***

Kouhei FURUYA*, Kosuke TAKATORI**, Osamu SONOBE*** and Teizo MABUCHI***: Occurrence of chalk brood disease in honey bee larvae in Japan

Summary

A previously undescribed fungal disease occurring in the larvae of honey bees was first found in March 1979 in Gifu Prefecture, Japan. The dead larvae were dried and somewhat mummified. Although most were chalky white to light brown, a few had dorsal surfaces covered with black spots. The larval bodies were compact masses of fungal mycelia, while the black spots were spore cysts. The causative agent was isolated on artificial media after having been taken from the larvae. The colonies on M40Y and MY20 agar media had surfaces deeply floccose and ranging from hyaline to dark gray. Spore cysts were produced in a fairly dense layer on the agar. There was a conspicuous sour odor. From microscopic observations, the disease was identified as *Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltoir, and thus represented the first case of chalk brood disease to be identified in Japanese honey bees.

Optimum vegetative and sporulative growth occurred at temperature ranges of 25–35 C and 30–35 C, respectively. Such temperatures seem to represent physiological adaptation to the environment, because the temperatures inside the beehives were maintained at 30–35 C by the bees themselves. A medium of malt extract-yeast extract agar supplemented with glucose or honey at concentrations between 5 and 20% (w/v) supported growth and sporulation of *A. apis*. Only slight growth and sporulation were observed when the glucose or honey reached the 30% level. With respect to NaCl tolerance, the fungus grew well and sporulated on an agar medium containing NaCl concentrations from 0 to 3% (w/v). At a 5% concentration, growth was very reduced and sporulation did not occur.

ハチノスカビ (*Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltoir) によるミツバチ幼虫の被害の記録は，1912年に Betts によってイギリスで報告されたのが最初で，次いで1913年 Maassen によって“Kalkbrut”病と名付けられて発表された (Skou, 1972)．その後，ヨーロッパ各地にも被害が確認されたが，近年になって北アメリカにも本菌による被害が及ぶに至

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った．すなわち，野生のハキリバチ類（*Megachile inermis* など）およびミツバチ（*Apis mellifera*）に本菌による被害が発生し（Melville and Dade, 1944; Baker et al., 1968; Hitchcock, 1972; Hitchcock and Christensen, 1972; Gochnauer and Hughes, 1976)，アメリカではすでに大部分の州に被害が及んでいる（Menapace and Wilson, 1976).

一方，わが国では1959年小林義雄博士によって本菌をはじめとする *Ascosphaera* 属の文献的な紹介が発表されているが，本菌によるミツバチの被害については認められていなかった（小林, 1959; 青木，1957)．その後，宇田川らによって秋田県産およびカナダからの輸入蜂蜜から本菌が分離報告された（Udagawa and Horie, 1974; 山崎ら，1975)．こうしたことから，ミツバチの *A. apis* による chalk brood disease が日本国内の養蜂場でも発生している可能性が大きいことは充分予測されていたが，果して1979年の春に岐阜県下で発生したミツバチ幼中のカビによる病気を調べたところ，*A. apis* による chalk brood disease であるとの結論を得たので以下にその概要を報告する．

Chalk brood disease の発生状況

1979年3月，鹿児島県からフェリーボートで運ばれてきたある養蜂場のミツバチを岐阜家畜保健衛生所においてミツバチの伝染病である腐蛆病の早期発見のため，家畜伝染病予防法に基づき検査を実施した．その結果，検査を行った100群中の3群にカビによる病気の発生を認めた．その後，北海道などから転飼してきた群にも同様の病徴が認められ，4月から5月にかけての定期的腐蛆病検査において，合計3,760検査群中280群に発生を確認した．なお，いずれの場合も腐蛆病の発生は認められなかった．

カビによって罹病死した若令蜂児（幼虫）は虫体の周囲を白色の石こう様物質で固められた状態で房室に固着しており，房蓋があるものも，ないものもあった．また，被害は比較的巣の周辺部に多発していた．死幼虫は石こう様にミイラ化し，多くは白色ないし灰白色であったが，少数ながら黒褐色のものも混在しており，いずれも独特の酸臭を発していた．

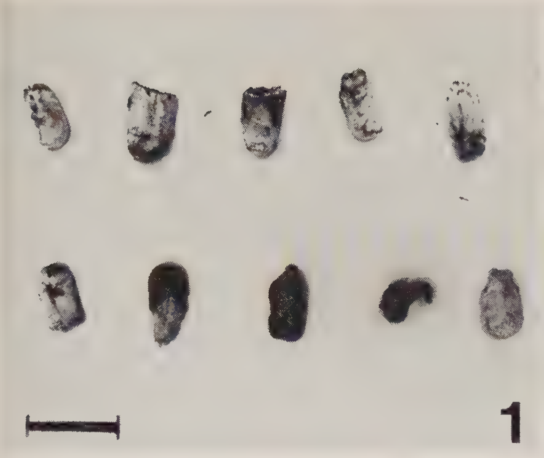
実験材料および方法

1）供試菌株：*Ascosphaera apis* SANK 11479，今回，岐阜県下で発生した chalk brood disease の幼虫から分離された菌株，*A. apis* NHL 2714，秋田県産の蜂蜜から分離され，国立衛生試験所から分譲を受けた菌株，*A. apis* No. 844，デンマークの Risø National Laboratory の Skou 博士から分譲を受けた菌株，以上の3株を用いた．

2）検鏡材料と原因菌の同定：罹病死した幼虫を採取し，顕微鏡観察に供した．原因菌の分離は，上記の幼虫上の菌糸および胞子を直接クロラムフェニコール（100 μg/ml）加 MY20，M40Y, PDA，オートミール麦芽の各寒天培地上に釣菌し，室温，自然光下で培養を行った．

3）栄養成長および子実体形成に対する温度試験：MY20 寒天培地を用い 20, 25, 30, 35, 40 および 45 C の各温度条件下で培養した．生育速度はコロニーの直径をもって測定した．また，子実体形成の度合は肉眼判定によった．

4）栄養成長および子実体形成に対する糖ならびに塩濃度の試験：基本培地（麦芽エキス 20 g，酵母エキス 5 g，寒天 20 g，水道水 1,000 ml）に糖としてグルコースならびに蜂蜜を濃度 5, 10, 20, 30, 40%（w/v）となるように，また，塩類として塩化ナトリウムを濃度 1, 3, 5, 7%



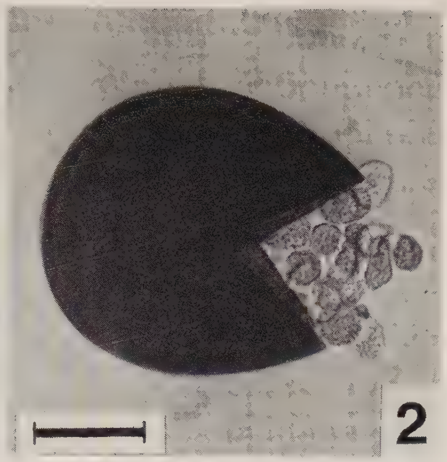


Fig. 1. Chalk brood diseased honey bee larvae removed from brood cells. (Scale: 1.0 cm)
Fig. 2. Spore cyst and spore balls of *Ascosphaera apis* (SANK 11479) isolated from infected honey bee larvae. (Scale: 30 μm)

(w/v) となるように加えて試験した．これらの培養は 30 C で行った．

結果および考察

1) 原因菌の形態と同定

罹病死したミツバチの幼虫のうち，石こう様，白色のものは表面全体が菌糸のみで覆われていたが，暗褐色ないし黒色のものでは子実体を多数形成していた (Fig. 1)．また，両者とも幼虫の体内に多数の菌糸が認められたが，子実体は体表上にのみ形成されていた．これらの検体から菌の分離を行った結果，供試した 4 種類の培地すべてに同一種が分離された．分離株は以下のような菌学的性質から *A. apis* と同定された．

Ascosphaera apis (Maassen ex Claussen) Olive et Spiltoir, Mycologia **47**: 242. 1955; Udagawa and Horie, Trans. mycol. Soc. Japan **15**: 105. 1974. (Fig. 2)

M40Y および MY20 寒天培地上，25 C での生育は，きわめて良好，やわらかい羊毛状，初めほぼ白色であるが，1 週間ほどで多数形成される子実体のために灰色–黒褐色になる．子のう果 (spore cyst) は密にかたまり，ほぼ表在性，球形–亜球形，40–100 μm，子のう果壁は暗褐色，半透明，やや粗面，内部に多数の胞子球 (spore ball) を生ずる．胞子球は球形–亜球形，直径 8–15 μm．子のう胞子は無色，1 細胞，2.8–3.5 × 1.2–2.0 μm，平滑，長楕円形，やや湾曲するものもある．菌糸は隔壁を有し，無色，平滑，幅 5–7 μm．分生子は形成されない．

オートミール麦芽寒天培地上での生育は，上記の培地より劣るが，子実体は充分に形成される．37 C での生育は 25 C の場合よりも，むしろ良好であるが，子実体の形成はやや劣る．

いずれの培地においても特有の酸臭を発するが，とくに M40Y 寒天培地において顕著である．

Ascosphaera 属は Ascosphaerales, Ascosphaeraceae に属し (Ainsworth, 1971; Skou, 1972)，これまでに 5 種が知られているが (Skou, 1972, 1975; Skou and Hackett, 1979; Spiltoir and

Olive, 1955)，いずれもハチ類の幼虫に寄生ないし腐生的（排泄物などに）に見い出される特異な生態の菌である．

2) 栄養成長および子実体形成に対する温度試験

栄養成長に対する適温は菌株により多少異るが，30–35 C の範囲であった (Fig. 3)．また，子実体の形成は 25–35 C の範囲で認められたが，なかでも 30 C が最も良好であった． 20 C および 40 C では生育が認められても，子実体の形成は全く認められなかった．

以上のことは本菌の生態と考え合わせると大変興味のある知見と思われる．すなわち，一般にミツバチの巣の中は年間を通じて，30–35 C に保たれているからである． その温度調節法として，夏にはハチ自身が巣箱内に水を運び入れ翅で風を送り，水の気化作用で温度を下げ，冬には巣内の働きバチが多数集まって蜂球を作り，熱の発散を少なくするばかりでなく，貯蔵蜂蜜を飲んで自ら体内発熱をすることが知られている（岡田，1975）． したがって，本菌が巣内の幼虫に寄生するにはきわめて好都合な温度条件と考えられる．

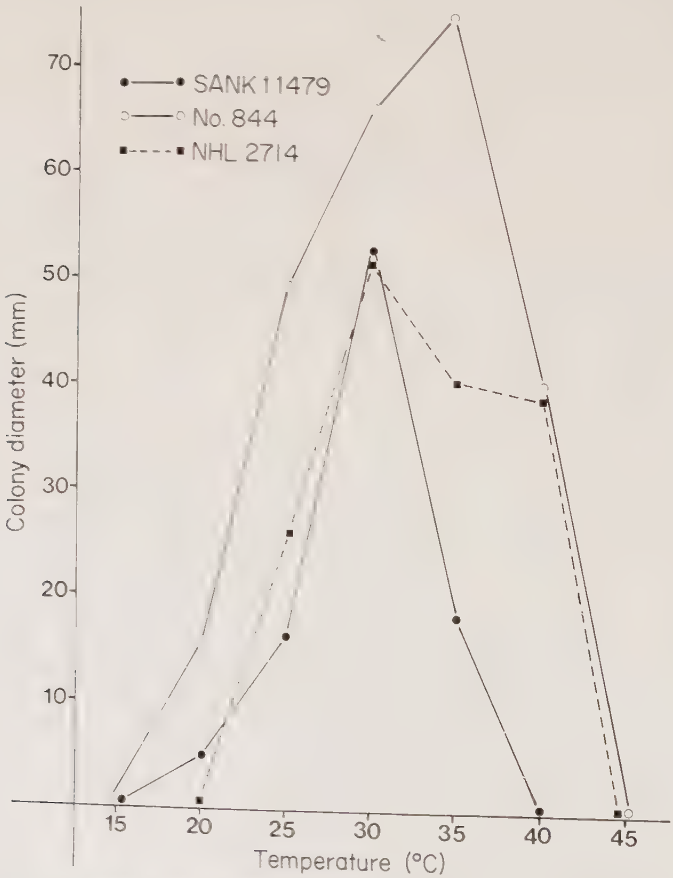


Fig. 3. Growth of *Ascosphaera apis* on MY20 agar medium after 4 days' incubation at 7 different temperatures.

3) 栄養成長および子実体形成に対する糖ならびに塩濃度の試験

罹病死したミツバチ幼虫からの本菌の分離に際して，M40Y や MY20 寒天培地のように高糖濃度の培地が栄養成長，子実体形成ともに適していることがわかった．そこで，本菌の栄養成長および子実体形成に対する糖濃度の影響を調べた．また同時に，塩化ナトリウムに対する影響も調べた．その結果，本菌の栄養成長にはグルコース濃度も蜂蜜の濃度もともに 5–20%(w/v) の範囲が良好であった (Fig. 4)．また，子実体の形成に対しても，栄養成長の場合とほぼ同様に10–20%の範囲で良好な結果を示した．グルコースおよび蜂蜜の濃度 30%では生育はきわめて抑制されたが，子実体はごくわずかに形成した．しかし，濃度40%での生育は全く認められなかった．

一方，塩化ナトリウムに対しては，濃度 0-3% (w/v) の範囲ではよく生育し，また，子実体の形成もみられたが，5%の濃度では生育は著しく阻害され，子実体も全く形成されなかった．

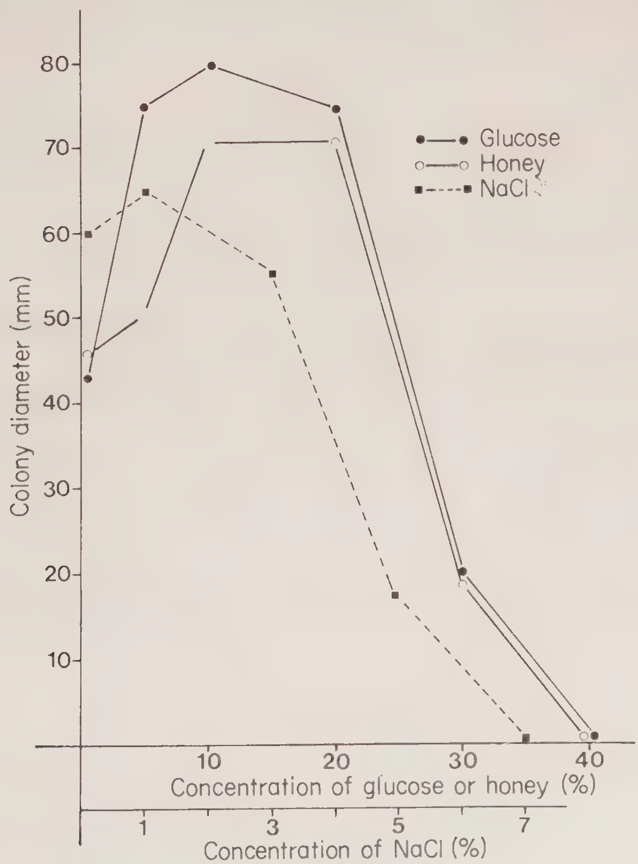


Fig. 4. Growth of *Ascosphaera apis* (SANK 11479) on malt extract-yeast extract agar medium containing different concentrations of glucose, honey or NaCl after 7 days' incubation at 30 C.

ま と め

以上のように，今回岐阜県下で発生したミツバチ幼虫の被害は，欧米ですでに広く蔓延している *Ascosphaera apis* による chalk brood disease と断定した．本菌の生育温度は30C 前後であり，耐塩性は高いとはいえないが，高糖濃度下での生育状況から，むしろ好浸透圧性菌と考えられる．本種がどのようにして日本国内に伝播されたかは明らかではないが，越冬時のミツバチに与える輸入蜂蜜から持ち込まれた可能性が大きいものと考えられる．あるいはまた，日本の野生ミツバチ (*Apis indica*) には以前から chalk brood disease があって，それがたまたま養蜂場のミツバチ (*Apis mellifera*) に伝染したとも考えられる．

Chalk brood disease の予防および治療には thymol や formalin あるいはまた，各種の抗カビ性抗生物質の適用が試みられている (Hitchcock, 1972)．また，最近 citral および geraniol が *A. apis* の生育を阻止することも報告されているが，まだ実用化されてはいないものと思われる (Gochnauer et al., 1979).

終りに，本研究に際し御指導を賜わった国立衛生試験所宇田川俊一博士，玉川大学教授岡田一次博士および菌株を供与されたデンマーク Risø National Laboratory の J. P. Skou 博士に感謝の意を表します．

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山崎幹夫，堀江義一，宇田川俊一，越後多嘉志，君　政子．1975．はちみつの中の菌類分布について．食衛誌 **16**: 1–6.

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新 刊 紹 介

Domsch, K. H., W. Gams, and Traute-Heidi Anderson: Compendium of Soil Fungi. Vol. 1, 859p, and Vol. 2, 405p. Academic Press, London. 1981. US $ 192.50 (£ 80.00).

本書は2巻からなり，著者らの前著 Pilze aus Agrarböden (1970) ［英訳版 Domsch, K. H., and W. Gams: Fungi in agricultural soils (1972)］を拡大，充実化したものである．すなわち，前著では209種の土壌菌類を扱った内容であったが，今回は約400種が収載されている．配列は分類，生態，生理の各セクションに並べられ，オランダの CBS の Gams 博士が担当された分類の項では学名，主要シノニム，記載の出ている文献名，簡単な記載，形態を示す線図または写真，関連種との相違，細胞学的知見，遺伝，微細構造，細胞構成成分，DNA 分析が述べられている．

一部の図は前著のものをそのまま用いているが，今回用意された形態図はきわめて多数であり，特筆される点はノマルスキー微分干渉顕微鏡および走査電子顕微鏡による写真が新らたに加えられたことにより，極微細構造の識別をはかったところである．また，*Acremonium* (11種)，*Aspergillus* (24種)，*Chaetomium* (10種)，*Fusarium* (23種)，*Mortierella* (19種)，*Mucor* (10種)，*Penicillium* (51種)，*Phoma* (10種)，*Pythium* (10種) など，とくに重要な土壌菌類については検索表をつけて同定の便をはかっている．

前著以上に図，写真の出来生えは素晴らしく，読者の目を楽しませてくれる．また，第2巻は属の検索と6,593に及ぶ文献を収容している．とくに検索表の脇に簡単ながらポイントになる形態図を配しているのはアイデアであろう．

この2冊の成書には土壌菌類を扱う菌学者，植物病理学者，医真菌学者，生態学者にとって貴重な情報がつまっている．最も普通に温帯，熱帯地域に分布する重要な菌類を網羅しているので，本書をひもとくことによってかなりの範囲の土壌糸状菌を容易に同定することが可能であろう．ただし，例えば *Anixiella*, *Ascodesmis*, *Boothiella*, *Neurospora* などまだまだ本書に述べられていない土壌菌類も多いところを十分注意する必要がある．学名の適用は大体において妥当であるが，*Aphanoascus* のように疑問を感じる属名を採っている箇処もある．とくに *Penicillium* の種名の検討は不十分であり，*P. steckii* や *P. rubrum* を項目として挙げるのはどうかと思われる．一方，*Aspergillus* については *A. ochraceus* を *A. alutaceus*, *A. tamarii* を *A. erythrocephalus* とそれぞれ改めるなど従来の扱いと異なる部分もあって，著者の見解をさらに詳細に知りたいものと思う．

生態の項では分布についても相当よく調べられており(日本の分布については記載もれが目立つが)，生理では菌の生育温度条件などを始め興味ある諸性質が述べられている．これらの情報は応用菌学の分野においても，大変役立つものと考えられる．著者らのアルバイトに敬意を表したい．

(Shun-ichi Udagawa　宇田川俊一)

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資　　料

愛媛県における高等菌類（キノコ類）の研究史とその種類

沖　野　登 美 雄*

Tomio OKINO*: Research history and species of the higher fungi in Ehime Prefecture

ここ数年各地で発行されている植物誌のなかにも，高等菌類がとり入れられるなど，キノコ類の研究もさかんになってきた．著者の住む愛媛県のキノコ類の研究史と，いままでに報告された種類を調べてみた．

研究史　著者が調べた限りの文献では，植物学雑誌（以下植雑）30巻11号に，掲載された安田　篤 (1908-1924)：菌類雑記 (57) の報文中に，キヌガサタケの記事がある．採集月日の記載はないが，「伊予国松山ニ産ス。小松崎三枝氏，恵与ニ係ル」と書かれている．

また，今関六也（1939 a）は日本産マンネンタケ属の報文の中に，マンネンタケの産地を "Onsen-gun, Ehime Pref. (Yamamoto, H., Aug. 1916)" と記載している．これらのことから，愛媛県における研究は大正5年 (1916) ごろからではないかと考える．

当時報告された種類　植雑22-38巻に安田　篤：菌類雑記 (1-147) という記事がある．この菌類雑記57-105 (30-34巻) 中に，愛媛県を採集地とするキノコ27種類を拾いあげることができる（表1）．その中の2種は報告によると本県が日本および世界での最初の発見地となっている (Yasuda, 1917a, b)．すなわち，日本で最初に発見されたエヒメウスバタケ (*Irpex iyoensis*) と世界で最初に発見されたガマタケ (*Polyporus komatsuzakii*) である．前者には "Matsuyama; 17 Sept. 1916 (Hajime Yamamoto)"；後者には "Isozaki-mura, Kita-gori; 10 Dec. 1916 (M. Komatsuzaki)" と記されている．なお，後者の Isozaki は Ikazaki（喜多郡五十崎(いかざき)町）の誤りと思われる．また，その後の報告では愛媛県にクジラタケ (*Polystictus orientalis*) が産することを記録している (Yasuda, 1922)．文中に "Matsuyama, Prov. Iyo; 15 Sept. 1916 (H. Yamamoto)" とあり，山本　一氏が安田　篤氏に送ったことがわかる．

以上は安田 (1908-1924) による記事であるが，今関 (1940)：日本産 *Hymenochaete* 属の研究報告中にマツノタバコウロコタケ (*H. yasudai*)〔南宇和郡日荘町（これは御荘(みしょう)町の誤り），Oct. 1922, (Ogata-M)〕をはじめエビウロコタケ (*H. rubiginosa*) とミヤベオオウロコタケ (*H. intricata*) の記録がある．また，今関 (1939 b) はさらに *Stereum* 属3種を愛媛県から記録している．

そのほか，川村 (1954) もコイッポンシメジ (*Entoloma prunuloides*) とナガエノアカチャワンタケ (*Sarcoscypha floccosa*) を愛媛県から記録している．

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表1．菌類雑記（安田　篤）に報告されている愛媛県産キノコ類

和名	学名	植雑 巻，頁	備考*
キヌガサタケ（一名コムソウタケ）	*Dictyophora phalloidea* Desv.	**30**：395	*D. indusiata*
ヒイロチャワンタケ	*Peziza aurantia* Müll.	**31**：41	*Aleuria*
アカスズメノワン	*Lachnea scutellata* (L.) Gill.	**31**：75	
エヒメウスバタケ	*Irpex iyoensis* Yasuda	**31**：74	*Hydnochaete ravenelii*
ヒメシワタケ	*Merulius molluscus* Fr.	**31**：170	
ツメタケ	*Pleurotus griseus* Peck (=*Agaricus griseus*)	**31**：198	疑問種扱い
ハスタケ	*Trametes ungulata* Yasuda	**31**：244	*Gloeophyllum ungulatum* ミダレハスタケ
カタアナタケ	*Poria callosa* Fr.	**31**：245	*Trametes serialis* ダンアミタケ
カンゾウタケ	*Fistulina hepatica* (Huds.) Fr.	**31**：245	
ガマタケ	*Polyporus komatsuzakii* Yasuda	**31**：329	*Fomitopsis*
シロアンズタケ	*Cantharellus pallidus* Yasuda	**32**：49	
ツチウロコタケ	*Stereum epigaeum* Yasuda	**32**：75	*Vararia epigaea*
ムラサキウスバタケ	*Irpex purpureus* Yasuda	**32**：173	*Hirschioporus*
アカキクラゲ	*Dacryomyces aurantia* Schw.	**32**：174	*D. aurantius*
チャアナタケ	*Poria inermis* Ellis	**32**：263	*Fuscoporia*
コガネウスバタケ	*Irpex tabacinoides* Yasuda	**34**：96	*Hydnochaete*
レンゲタケ	*Polyporus floriformis* Bres.	**34**：162	*Tyromyces*
スズメタケ	*Polyporus rhipidium* Berk. var. *pusillus* (Pers.) Lloyd (=*Polyporus pusillus* Pers.)	**34**：194	*Favolus rhipidium* var. *pusillus*, *Dictyopanus pusillus*
カワラタケ寄生菌	*Hypomyces aurantius* (Pers.) Tul.	**34**：294	
ウスキアナタケ	*Poria pulchella* Schw.	**34**：321	*P. medulla-panis*
シロヒメカイメンタケ	*Polystictus cuneatus* Ellis	**35**：66	*Hapalopsis* ヒメシロカイメンタケ
ニクアナタケ	*Poria ornata* Peck	**35**：205	疑問種扱い
マツノサルノコシカケ	*Fomes fastuosus* Lév.	**35**：221	*Cryptoderma* センダンサルノコシカケ
オツネンタケモドキ	*Polyporus brumalis* (Pers.) Fr.	**36**：82.	*Polyporellus*
ヒメハウロコタケ	*Trametes purpurea* Cooke	**36**：102	
ヒラフスベ	*Calvatia versispora* Umem.	**37**：203	*Laetiporus versisporus*
ホオヅキタケ	*Entonaema liquescens* Möll.	**38**：46	*E. splendens*

* 伊藤（1955，1959）：日本菌類誌などの扱い．

菌類目録　愛媛県の菌類目録について，菌類単独のものは発見できなかったが，八木(1928)の著書「愛媛県植物誌」の凡例中に，本県産菌類を小松崎三枝氏と共同で記載している．それによれば，そう菌類12種，子のう菌類31種，担子菌類163種の記録がある．この中からいわゆるキノコの種類を拾いあげてみると，子のう菌類が2種，担子菌類が110種あった．本県では，これが最初の菌類目録と思われる．

これとは別に，Yasuda (1917c) の記事がある．その中に，産地が Iyo（伊予＝国名）とするものを85種類数えることができる．

このことから，キノコ類の一部の種類（主にヒダナシタケ目）であるが，1917年（大正6年）にはすでに85種類を愛媛県から採集，記録していることがわかる．

当時の採集者　緒方松蔵，小松崎三枝，八木繁一，山本　一の各氏から今関六也，川村清一，安田　篤の各氏に標本を送り同定を依頼していたことが，八木(1928)，安田(1908–1924)，Yasuda (1917a, b, c)，今関 (1939 a, b; 1940) などの記事によってわかる．

八木，山本両氏は，県内で知名の人である．山本　一氏は明治7年に周桑郡丹原町で生れ，明治30年に愛媛師範学校を卒業して，周桑郡国安の高等小学校の先生となり，明治34年文検をとり，翌年松山商業学校が新設開校したときに，同校の博物および国語の先生として赴任した．明治37年，高知師範学校へ転任し，ここで5年間，その後中村中学校（現在の土佐中村市）に8年間勤めた．

その後，再び松山へ帰り，大正4年済美高等女学校，大正7年—昭和12年まで，西条中学校で博物を教えていた．

牧野富太郎博士を師として，特に愛媛，高知両県を中心に植物を研究していた．ヤッコソウの発見者として知名であったが，昭和42年松山市で亡くなった．

八木繁一氏は明治26年に松山で生れ，大正3年愛媛師範学校を卒業し，上浮穴郡抑谷村の小学校訓導，その間に文検をとり，大正9—10年余土付属小学校訓導（松山市），大正11年—昭和16年まで愛媛師範学校教諭として博物学を教え，愛媛師範学校から現在の松山東高等学校，余土中学校，松山北高等学校などに勤め，昭和31年に退職され，この年に県立博物館の設立に専念した．

植物全般にわたって研究をされたが，やはり主力は瀬戸内海から豊後水道にかけての海藻の研究である．なかでも淡水紅藻オキチモズクの発見者として知名の人であり，著書も多いが紙面の関係で省略する．昭和55年6月に亡くなった．

緒方松蔵氏は三好(1965)の論文によると，「宮崎県人で大正のころ南予（南宇和郡）へきて，昭和8—9年ごろまで，南，北宇和郡各地の小学校の先生をしていたが，その後再び宮崎へ帰った」と記してあり，また「南予を中心にシダ以上の植物研究をしていた」とある．

同氏の南宇和郡植物誌(1926)にはシダ類119種，種子植物類1,160種を載せていると付記している．また薬草にも通じ，貝類の研究もされたとのことである．また，オガタザラという貝もあると聞く．

小松崎三枝氏は，やはり三好(1972)の論文によれば，「水戸の人で茨城師範を出て，小学校の先生をしていて文検をとり，水戸の水産学校の先生になり，明治40—41年ごろ愛媛師範にきて，大正7年に滋賀師範に転任した」と記してある．氏は水産学が専門で，水戸の水産学校のころ，水産動物学という教科書を著わしたとあり，愛媛県に移ってからも，水産学を専門に教えていたようで，とくに貝類の研究家として知名の人であった．

八木氏が海藻類研究のかたわら，貝類にかなり関心を持っていたのも小松崎氏の影響が大きいとも付記している．

このように県下の菌類研究は他の専門分野を研究するかたわら行われていたが，とくに小松崎三枝氏は県下での高等菌類研究の草分けとなった人である．

その後の報告と課題　著者は，昭和34–36年に，当時愛媛大学農学部学生，のち面河中学教諭となった中本　司氏と共著(1959–1961)で，愛媛県に産するキノコI–VIと題し，229種の採

集を報告したことがある.

しかし，当時小松崎氏などのことは調べていなかった．その後，諸先輩の文献からエヒメウスバタケ (*Irpex iyoensis*=*Hydonochaeta ravenelii*) やガマタケ (*Polyporus komatsuzakii*=*Fomitopsis komatsuzakii*) などの記録を知ることができた．しかし，著者は今日に至るまでこの両種については再度の採集による確認を行なっていない.

地方でキノコを研究する者として，県下の過去におけるこれらの記録を，不完全ではあるが，公式の場で書きとめておく必要があると思い，あえて筆をとった次第である.

本報告にあたり，色々と文献の便を計っていただいた，今治明徳短大　山本四郎，北条聖カタリナ短大　三好保徳，松山東雲短大　松井宏光の各氏に感謝します.

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資　　料

菌類民俗学と歴史資料 (8)

小　林　義　雄*

Yosio KOBAYASI*: Materials on ethnologic and historical mycology (8)

17. キヌガサタケ類 The genus *Dictyophora*

真菌類中で最も分化し，したがって多くの分類学書では最も高等な位置に格付けされており，かつきわめて美しく，分布上から見ても生理的にも特徴のあるこのキヌガサタケ類を歴史と民俗学の面から大観することは無意味ではないと思う．ヨーロッパはこれの本来の産地ではないため古い文献はなく，ヨーロッパ人が海外へ科学調査をはじめてからようやくこれの存在が知られるようになった．しかし，今から千年以上も昔に中国ではすでにこれに関すると思われる記録が残されてあったことは真に驚異である．わが国では170年昔にこの菌の紹介があり，続いて美しい図が発表された．

史上最古の文献と思われるもの

それは中国の唐代に著わされた段成式 (Twan-ching-shih ?–863) 著酉陽雑俎(ユウヨウサツソ)である．前集20巻と続集10巻計30巻よりなり唐代宮廷内の諸書を読み，動植物，道教，衣食，風習などを述べた随筆である．その第19巻に次のような記事が載っている．

原文（国立文書館蔵　元禄10年日本復刻版）

又梁簡文延香園大同十年竹林吐一芝長八寸頭蓋似雞頭実黒色其柄似藕柄内通幹空　皮質皆純白根下微紅雞頭実処似竹節　脱之又得脱也　自節処別生一重如結網羅四面同可五六寸円繞周匝以罩柄上相遠不相着也　其似結網衆目軽巧可愛其柄又得脱也　験仙書与威喜芝相類.

和訳（小林訳）

西魏の大統10年 (A.D. 544) に唐の文帝の所有する延香園の竹林に一奇菌が発生した．菌体の長さ8寸 (20 cm)，頂に雞頭状，黒色の蓋があり，その姿はオニバスの果実に似ている．柄は蓮根のごとく中空で，その基部は淡紅色であるが，その他は白色である．頭部の基に竹節に似て可動性の節があり，この節部から一枚の網細工が形成されている．その周囲は5～6寸 (14–5 cm)，釣鐘状で柄を囲み，その縁は柄からずっと離れている．この網細工はきわめて美しく愛らしいもので，柄から取りはずすこともできる．これは道教でいう威喜芝に相当する．

英訳（南方熊楠．1894．Nature **50** No. 1281；平凡社版全集第10巻欧文篇 pp. 35–36）

In the 10th year of the period (Tá-Tùng 544 A.D.) a fungus grew in Yen-hiáng Gardens owned by the Emperor Kién-Wan. It was eight inches long with a black head resembling the fruit (that is, the Torus) of *Euryale ferox* (オニバス), stem hollowed through inside the root of *Nelumbium speciosum* (ハス), skin all

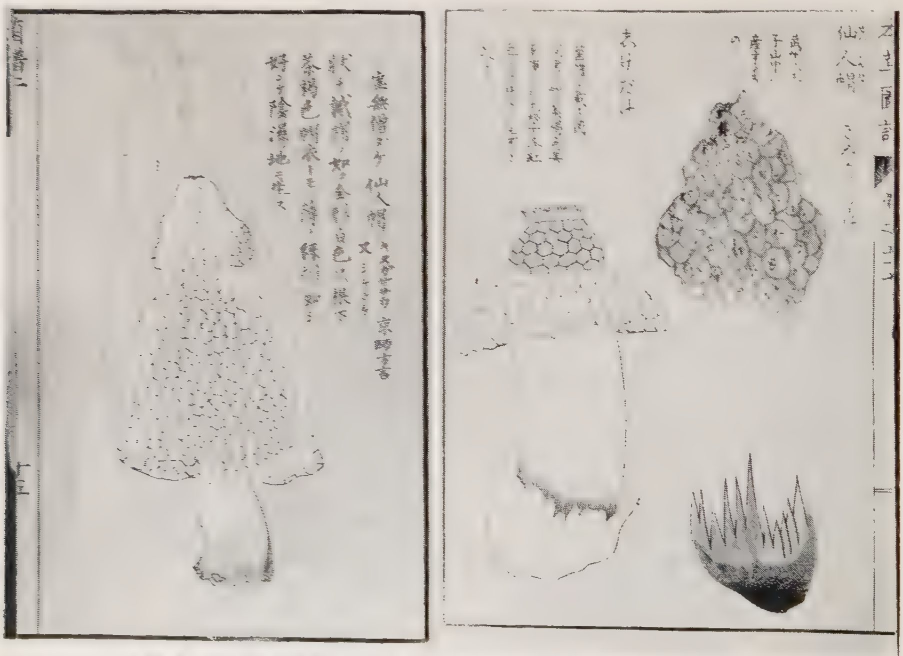
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white except below the root, where it was slightly red. Portion like the fruit of *Euryale* had below a joint like that of the bamboos, and was removable; from the joint a sheet was developed, simulating a network, five or six inches in circumference, surrounding the stem in the manner of a bell, but distant and separate from it. The network was fine and lovely, and also removable from the stem. It is allied Wei-hi-chi (the Auspicious fungus of Graveness and Pleasure) of the Taoist writings.

[illegible]を指すことに間違いないと確信しているが，読者は[illegible]．なお，この[illegible]の芝，紫芝，夜光芝などの興味深い記事[illegible]である．

日本の本草書に載るもの

[illegible]以前の文献には載っておらない．菌類図譜として体をなした最初[illegible]までもない．19世紀に至ってはじめて増[illegible]された．これには梅雨後に諸処の陰湿地にま[illegible]（漢名）などの名を挙げ，また蔵器，雨[illegible]している．次に1835年に刊行された坂本浩然の菌譜（第[illegible]っている．けだし虚無僧（コムソウ）の網笠に由来して命[illegible]，ニマガササウ（京師方言）が示されている．シケダケ[illegible]．また[illegible]当時から150年経た現在でも本菌が竹藪など[illegible]を欠くが，次頁の一種シケダケとして図示してあるものに[illegible]でおり，実物から写生した[illegible]確かである．本書に次いで出版された岩崎灌園の本草図譜（1844）の菌部4



[illegible]本草図譜に載る図．

にキヌガサタケと称する２品が並んでいる．右図はスッポンタケ，左図は菌史[illegible]てある．よく見ると[illegible]．また，[illegible]この菌に充てられ，右頁の左図と左頁の右図はその命名とともに菌史から転載した[illegible]．左頁右図は仙人帽（キヌガサタケ），武州八王子で採ったと記してあるが，マントの存在が不明である．なお，この本草図譜は浩然の図譜より以前に完成されたことになってお[illegible]に不審を抱く方もおられるかも知れぬが，菌類に関する部分は著者灌園常正の死後，[illegible]弟子と協力して補ったもので，もちろん浩然以後の作品である．この菌は稀品で[illegible]小石川の竹藪で採られ，小倉博士は東大構内の旧前田邸内で見たといわれた．

欧州人による研究史

最初の記述はオランダの Rumphius によるものである．アンボイナ[illegible] *Phallus daemonum* Rumph. の名で[illegible]である．これが現在キヌガサタケ（*Dictyophora indusiata*）の[illegible]名になっている．この種名の[illegible] Pere Vaillant [illegible]オランダ[illegible]



図 2. 左．[illegible]（*Dictyophora rochesterensis*），右．Rumphius 著アンボイナ[illegible]

これ，Ventenat (1798) によって発表され，Persoon (1801) によって引用された *Phallus indusiatus* である．それ以来多くの探検家達により熱帯各地で採集，勝手に命名され，その数は20に及んでいる．しかし，よい図がなかったのは生品から直接に画く機会が少なかったためであろう．

Gaudichaud (1826) の探検報告書に掲げられたものは傘の外側からマントが生じているようにみえ，模様化された想像図に過ぎない．一般に引用されている図は A. Möller (1895) が南ブラジルのブルーメナウで採った菌に基づくが，しかし必ずしも本種の典型的な形ではない．今までの菌類書にはこの図が多く使用されていた．最近イギリスの Reid (1977) が西インド産の２種 *D. multicolor* var. *laeticolor* と *D. merulina* とを美しい水彩画で発表し，ようやく欧米人にも身近に感じるようになったようだ．すでに切手の図案にも選ばれ，台湾とアフリカのザイール[illegible]されている．

食品としてのキヌガサタケ

劉慎諤ら (1936) は中国産のものをはじめて菌学的に記載し，マントの黄色のものを新種 (*D. lutea*) として発表したが，彼によると食品に供されるものには，*D. indusiata* のほかに *D. duplicata* もあるという．食品名としては竹蓀，竹生，竹参などがある．四川，貴州，雲南が産地でとくに雲南産が上品である．傘と壺とを取去り，数本を[illegible]ったものが食品である



[illegible] キヌガサタケ．[illegible] B．乾燥食品としての竹蓀．

が，乾きすぎると湿らし，変質を防ぐために硫黄でくん蒸する．また料理法については中国名菜譜（日本版あり）にも載っており，故篠田統氏が日菌報 **11**: 115 (1970) にその数種を紹介している．

美の対象と生長の速度

アンドン型に拡ったレースのマントの美しさは初めてこれをみる者の心を捕らえる．洋人はこれを crinoline または lace fungi と通称している．前名は婦人のスカートのふっくらと拡がった形に由来する．マントの色が黄色の品種はわが国中南部から中国に分布し，淡紅色の品種はさらに南のマレー諸島を中心にセイロン，ニューギニヤに分布する．別にニューギニヤには多色の美しいものが見いだされており，*D. multicolor* と称する．かつてニューギニヤ在住の画家 Rowan 夫人がこれらを4枚の水彩画にまとめ，メルボルンの女性菌学者 McLennan によって1932年に発表された．マントの色が黄，淡紫，紫褐色，緑に色分けされた．私も白，紅，黄色の品種をカラーで発表 (1965) したことがある．このマントの伸び方は植物界中で最も生長の速いものであると色々な書物に記されている．わが国でも今関六也 (1933)，衣川堅二郎 (1960) その他の諸氏の観察があるが，これは単なる生長ではなく，菌蕾の状態で，内部の茎やマントの組織は増大，圧縮状態になり，菌蕾の破壊と同時にこれらが伸長するのであって，いわば畳まれたアコーディオンがぱらぱらと伸びるに等しい．

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Reid, D. A. 1977. Some Gasteromycetes from Trinidad and Tobago. Kew Bull. **31**(3): 657–690, col. pl. 22, 23 (*D. merulina* and *D. multicolor* var. *laeticolor*).

正 誤 表：21巻2号目次および総目次

21巻	誤	正
2号目次	資料・速報・総説	資料・速報
総目次II	資料・速報・総説	資料・速報

日菌報 (Trans. mycol. Soc. Japan) **22**: 145–146, 1981

新 刊 紹 介

Hoog, G. S. de and E. J. Hermanides-Nijhof: The Black Yeasts and Allied Hyphomycetes. Studies in Mycology No. 15. 222 p., 1 pl. Centraalbureau voor Schimmelcultures, Baarn. 1977. 価格 50 ダッチギルダー.

1979年発行の Centraalbureau voor Schimmelcultures—75 years culture collection (新刊紹介—日菌報 **21**: 56. 1980.) と題する CBS 創立75周年記念の小冊子によれば, Dr. de Hoog は1971年に CBS の研究陣に加わり, 研究テーマは仮軸型分生子系に属する不完全菌と酵母様線菌類の分類である. 前者の主要業績としては, CBS Studies in Mycology No. 1 (1972): "The genera *Beauveria*, *Isaria*, *Tritirachium* and *Acrodontium* gen. nov. 41p." と No. 7 (1974): "The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov. 84p." である. 特に分類の理論と方法論に深い関心をもっている. もう一人の Drs. Hermanides-Nijhof は1960年に CBS のスタッフとなり, 現在コレクションの事務的業務に加えて, 特に *Cylindrocarpon*, *Aureobasidium* の分類学的研究に従事している.

さて, 本書は黒色酵母とその類縁線菌類に関するモノグラフであり, 3つの部分(原論文)より構成されている. 第1章 *Rhinocladiella* and allied genera, by G. S. de Hoog (pp. 1–140); 第2章 *Aureobasidium* and allied genera, by E. J. Hermanides-Nijhof (pp. 141–177); 第3章 Survey of black yeasts and allied Hyphomycetes, by G. S. de Hoog and E. J. Hermanides-Nijhof (pp. 178–222).

第1章では, *Rhinocladiella* 様不完全菌の多数の培養株とハーバリウム標本の形質について, 伝統分類学 (比較形態学) 的研究ならびに化学分類学的分析が, その道の専門家の協力を得て展開されている. さらに前者によって得られた形態学的データの 106 の形質のマトリックスにもとづいて数値分類学的アプローチが試みられている.

材料と方法の項には用いた数値分類法と Pyrolysis-mass spectrometry (PyMS) の分析法が図解入りで詳述されている. 形態学的データのマトリックスの項には, 菌糸体の特徴, 分生子形成細胞の分化の程度, 分生子形成法 (単純分芽, パーカレント, フィアロの3様式) が類型化, 図解されている. 加えて, 数値分類学的解析に用いた106の形質・属性の一覧表が示されている. 形態学的データの数値分類の項では, 仮軸型分生子系 (sympodial states in *Rhinocladiella* and similar form-genera) とパーカレント型分生子系 (state with percurrent conidiogenesis in *Exophiala*) の時代をとる菌群について, 各々の菌群のデンドログラムと主成分分析による2次元マップが示されて, その相違が論じられている. また分生子時代を附随する子のう菌類の科レベルの分類体系がデンドログラムで示され, そのデンドログラム中に, 4つの形質の4組, 計16の形質について, その分布が視覚的に表現されている. この表現法は興味深い.

化学的データの項には, PyMS 分析の結果が, 量的比較は non-linear map, 質的比較はパイログラムにより図示されている. また代表的な2種の細胞抽出物中の炭水化物のガスクロマトグラムが図示され, 16種の細胞加水分解物中の単糖類, ポリオールの構成が一覧表にしてまとめられている. 章末に, 子のう菌類の目ごとに不完全菌の属名とそれに対応するテレオモルフの属名が付録として整理されている. この付録はテレオモルフーアナモルフの属レベルの関係を知りたいという読者にとって便利であろう.

本章で容認, 記載されている属は次の通り. *Zasmidium* Fr. emend. de Hoog (1種), *Leptodontium* de Hoog 新属 (5種, 1変種), *Ramichloridium* Stahel ex de Hoog 新属 (13種, 2変種), *Rhinotrichella* Arn. ex de Hoog 新属 (1種), *Rhinocladiella* Nannf. (4種), *Exophiala* Carmichael (7種, 2変種), *Phaeococcus* de Hoog 新属 (3種), *Rosulomyces* Marchand et Cabral (1種) および *Trichosporonoides* Haskin et Spencer (1種). 以上

の諸属に包含される41の種・変種につて，線画をつけて十分な培養的，形態学的特徴づけがなされ，かつ異名表，論議および研究に用いた培養株・標本についても詳しく記載されている．

第2章は，*Aureobasidium* と類縁属，すなわち *Aureobasidium* Viala et Boyer (15種，1変種)，*Hormonema* Lagerberg et Melin (3種)および *Sarcinomyces* Lindner (1種)のモノグラフである．著者はこれら3属を分生子形成法により再定義し，*Aureobasidium* は分生子の同調的形成により，*Hormonema* および *Sarcinomyces* は分生子の求基的形成により区別できると結論した．従って，かつて Ellis (1971), Kendrick and Carmichael in Ainsworth (1973) が *Aureobasidium* として記載，図示した菌は *Hormonema* であることが指摘されている．

第3章には，1，2章で容認，記載されたすべての属および類縁属の属レベルの検索表，関連の属名・種形容名・変種形容名の注釈付き照合表が収載されている．本章のなかで，8つの種レベルの新組合せが提案されている．

Wm. B. Cooke 博士が Mycologia 誌上で本書の書評 (Mycologia **69**: 1242–1244. 1977) を発表しておられるので，同博士のことばを借りて本書の感想としたい．

"Earlier monographic studies on genera with sometimes poorly defined morphological characteristics tended to lump groups of species now more readily separable on chemical as well as certain other morphological bases. This tends to bring systematic mycology more into the field of an exact science than an art." 同感!!

本研究で用いられているような総合的アプローチは今後，徐々に菌類分類学の領域に深く侵入，拡大していくものと思う．本研究の対象となっている菌群には，真菌症の原因菌や植物病原菌としてのカビもふくまれているので，その方面の研究者にとっても重要な文献となろう．

(Junta Sugiyama 杉山 純多)

日菌報 (Trans. mycol. Soc. Japan) **22**: 147–148, 1981

新 刊 紹 介

Hoog, G. S. de (editor): The Black Yeasts, II: Moniliella and Allied Genera. Studies in Mycology No. 19, 90 p. Centraalbureau voor Schimmelcultures, Baarn. 1979. 価格 25ダッチギルダー.

本書は，Studies in Mycology No. 15 ("The Black Yeasts and Allied Hyphomycetes") に続く，"黒色酵母"および類縁線菌類の分類に関する総合的研究のモノグラフである．7編のオリジナルの論文より構成されている．著者と論文タイトルをお示しすれば，本書の姿を捕えていただけると思う．

第1章 G. S. de Hoog: Taxonomic review of *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 1–36)

第2章 A. T. Martínez and G. S. de Hoog: Development and conidiogenesis of *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 37–49)

第3章 A. T. Martínez: Ultrastructure of *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 50–57)

第4章 A. T. Martínez, G. S. de Hoog and M. Th. Smith (in cooperation with P. Hogeweg and P. Bruinsma): Physiological characteristics of *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 58–68)

第5章 G. S. de Hoog and H. J. Roeymans (in cooperation with P. Hogeweg and P. Bruinsma): Volatile organic compounds produced by *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 69–75)

第6章 A. C. M. Weijman: Carbohydrate patterns of *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 76–80)

第7章 G. S. de Hoog: The taxonomic position of *Moniliella*, *Trichosporonoides* and *Hyalodendron* (pp. 81–90)

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本書で取り扱われている菌群は分節型分生子に加えて求頂的連鎖をするような分生子を生ずる暗色の酵母様線菌類である．第1章では *Moniliella* と類縁属の分類学的改訂を扱い，*Moniliella* Stolk et Dakin（2種，1変種），*Trichosporonoides* Haskin et Spencer（3種），*Hyalodendron* Diddens（1種）の3属を容認し，各属に包含される分類群について十分な記載，図解が収載されている．章末には関連の種および種以下の分類群の照合表が付されている．特に脂肪質の基質（バター，クリームなど）より分離された多数の *Moniliella* 様菌株のコレクションがこのような総合的研究を展開を可能にしたと，de Hoog 博士は序論の中で強調しておられる．

第2章では，*M. suaveolens* var. *suaveolens*, *M. suaveolens* var. *nigra*, *M. acetoabutens*, *T. madida*, *T. spathulata* および *H. lignicola* の菌糸と分生子の形成を time lapse 法により経時的に追跡し，それらの形成過程が詳細に記載，図解されている．大部分の分類群で，分芽型分生子の求頂的連鎖に加えて，出芽細胞の存在が確認されたこと，菌糸は成熟後にばらばらの細胞（分節型分生子）に分解しやすいことが述べられている．また二，三の記載用語が新しく定義されている．

第3章は，3属の隔壁孔と細胞壁の微細構造の電顕 (TEM) 的研究に関するものである．3属の供試菌株はいずれも小胞体 (ER) のアーチでおおわれたドリポア隔壁を備え，細胞壁の構造も不変的に担子菌型であり，出芽は全分芽的か内分芽的かのどちらかにより生ずることが確認された．

第4章～第6章には化学分類学的アプローチによる研究成果が披れきされている．まず第4章には，3属の一般的な生理的特徴づけが述べられている．すなわち，*Hyalodendron* は発酵能を欠き，トレハロース，メ

リビオース，ラムノースおよびイノシトールを利用する点で，*Moniliella* と *Trichosporonoides* の2属とは区別できるとしている．供試菌株の分離源の特徴が明らかに生理的行動に反映していることが指摘されている．そして DBB (Diazonium Blue B) 呈色反応，尿素の加水分解能によって示されているように，すべての菌株が担子菌系統である．生理的試験の結果にもとづいて，分類群間の類縁関係がデンロドグラムとして表現されている．

第5章には，3属の揮発性有機化合物について行われたガスクロマトグラフによる分析結果が述べられている．*Moniliella* と *T. spathulata* より構成される群は揮発性成分のパターンからおおよそ2つの群に分けられることがデンドログラムで示されている．

第6章には，3属の細胞酸加水分解中の炭水化物のガスクロマトグラムと単糖類・ポリオールのパターンが示されている．*Moniliella-Trichosporonoides* と *Hyalodendron* の間で，単糖類・ポリオールのパターンに顕著な相違がみられて，そのパターンから *Hyalodendron* は担子菌系統と考えられるけれども，他2属の所属位置は不確かであることが指摘されている．

第7章には，これら3属と関連する高次分類群との間における9つのキー・キャラクターの分布（割合）が視覚的に比較しうるような図に表現されている．これらの形質の分布は，3属すべてが担子菌系統であることを示唆しているけれども，二，三の主要な形質は既知の子のう菌時代の種類と共通する．

不完全菌類の分類について，最新の各種分析的技術を駆使して，総合的アプローチが展開された例は数少ない．前書ともども，本書は菌類分類学に新しい領域を切り開いた記念碑的業績になるものと思う．わけても，このような総合的研究を可能にしたのは，とりわけ de Hoog 博士の繊細な分類学的感性とすぐれた分類の理論・方法論に負うていることは勿論であるが，CBS の充実したカルチャー・コレクションやハーバリウムの伝統に加えて，化学分類屋もふくむ多士多才な CBS 研究陣の知的な財産と刺激があってのことであると思う．

（Junta Sugiyama　杉山　純多）